Influence of Red Cell Water Content on the Morphology of Sickling

By Margaret R. Clark, John C. Guatelli, Narla Mohandas, and Stephen B. Shohet

The response of sickle cells with varying water content to alterations in oxygen tension has been studied. Cells that were severely dehydrated while sickled retained the characteristic sickled morphology even after prolonged reoxygenation. When the cell water content was increased by reduction of the suspending medium osmolality, the cells unsickled. Cells that were dehydrated before deoxygenation were unable to assume the spiculated morphology typical of sickled cells. This was true both for high mean cell hemoglobin concentration (MCHC) discoid sickle cells and for irreversibly sickled cells. When such cells were resuspended in hypotonic medium before deoxygenation, they sickled with the characteristic morphology of sickle cells with normal MCHC. The morphological behavior of Ca-loaded sickled cells as well as irreversibly sickled cells showed a major influence of increased hemoglobin concentration and extremely high internal viscosity. Constraint on cell morphology by putative membrane rigidity was not observed.

The morphology of sickle cells can undergo many variations, depending on prior history as well as the partial pressure of oxygen to which the cells are exposed. For example, the irreversibly sickled cell (ISC) retains an elongated “sweet-potato-like” shape, even when fully oxygenated. ISC are thought to acquire this characteristic shape during previous prolonged or repeated sickling episodes. In addition, echinocytic non-ISC will produce “echinodrepanocytes” upon deoxygenation, and ATP-depleted sickle cells will not undergo morphological sickling.

In addition to the morphological changes associated with sickling, profound alterations in the cellular content of monovalent cations and water can also occur. The original observations of Tosteson et al. provided evidence for a transient cation leak during short-term deoxygenation. More recent studies by Glader et al. have demonstrated that prolonged sickling under certain conditions results in a permanent loss of total monovalent cations and water. In addition, they have shown that cell dehydration is essential for the generation of the morphologically distinguished ISC. Recently, we have begun studies intended to elucidate the effects of rapid dehydration on irreversible sickling. In this effort we have found that the morphological response of sickle cells to changes in oxygen tension is strongly influenced by the cell water content and mean cell hemoglobin concentration (MCHC). From the observations described below, we conclude that the sickle cell membrane, ISC or non-ISC, accommodates its form in a passive way to the geometrical organization of the hemoglobin inside, and that the hemoglobin organization itself depends on the cell water content.

MATERIALS AND METHODS
Preparation of ISC and Discoid Sickle Cell Subpopulations

Blood obtained from patients with homozygous sickle cell anemia was separated into subpopulations of ISC and mature discoid cells by centrifugation on Strattract II density gradients. The only modification of our previous method was to use continuous, rather than discontinuous, gradients, with a density range of 1.070–1.124 g/ml. As before, the gradients were formed on top of a dense Strattract cushion (1.144 g/ml) to prevent packing of the ISC against the bottom of the tube.

After removal from the gradient, cell samples from each subpopulation were added to an equal volume of 3% glutaraldehyde (prepared in 0.04 M Na phosphate buffer, pH 7.4) for determination of the percentage of ISC as before. Mean cell hemoglobin concentration was determined from centrifugal measurement of the hematocrit and spectrophotometric measurement of hemoglobin as the cyanomethemoglobin complex. The cyanomethemoglobin reagent contains 0.1% Triton X100 to ensure complete lysis of dehydrated cells, because saponin was not always effective in this regard. Because the variations in MCHC being studied were relatively large, the small differences in centrifugal red cell packing due to differences in MCHC were neglected.

Deoxygenation of Sickle Cells

For experiments requiring deoxygenation, cells were suspended in phosphate-buffered solutions of sodium and potassium chloride whose cation composition was varied as described below. N2 saturated with water was blown over the surface of the cell suspension with simultaneous agitation until the cells assumed the bluish-red coloration of deoxygenated hemoglobin. The flask containing the cells were then sealed to exclude air and placed in a shaking water bath at 37°C. At desired intervals, samples were withdrawn under an N2 atmosphere for morphological evaluation. Deoxygenated samples were placed directly into 3% glutaraldehyde with the minimum possible exposure to air. Samples to be reoxygenated were placed in tubes and agitated in room air. After 30 min, they were also fixed in glutaraldehyde.

Treatment of Cells Causing Retention of Sickled Shape

During the course of this study, deoxygenated sickle cells were subjected to three different types of treatment that resulted in the...
consisted of simple dehydration in hypertonic medium. This was of these three treatments was cellular dehydration. The first of 300 ± sickled shape. High-K medium contained 10 mM NaCl, 10 mM K These experiments were performed in both high-K and low-K sucrose simultaneously caused loss of water from the cells, tending with sucrose to the cell suspensions, as previously described.8 The solution of sucrose in buffer to suspensions of cells in isotonic content of the cells is reduced. In addition to the experiments in compensatory uptake of other ions. Thus, the total ion and water washes in warm medium restored the cation permeability of the remained cells by washing them in a medium identical to the final suspension medium but without Nystatin. The washing solutions were warmed to 37°C before addition to the cells because we had found that ice-cold medium failed to remove the Nystatin. Two washes in warm medium restored the cation permeability of the Nystatin-treated cells to normal.

The third treatment used in these experiments was the addition of the Ca ionophore, A23187,16 to cells in Ca-containing solutions. As was first shown by Gardos,15 accumulation of Ca by red cells in K-poor medium produces a specific loss of internal K without compensatory uptake of other ions. Thus, the total ion and water content of the cells is reduced. In addition to the experiments in K-poor medium, we also performed experiments in high-K medium to prevent water loss. The high-K medium contained 10 mM NaCl, 10 mM glucose, 10 mM K phosphate, pH 7.4, and 131 mM KCl, with a total osmolality of 290 ± 5 mosmole/kg. The low-K medium contained 5 mM KCl, 10 mM glucose, 10 mM Na phosphate, pH 7.4, and 134 mM NaCl, with a final osmolality of 290 ± 5 mosmole/kg. Sickle cells at 5% hematocrit were deoxygenated in isotonic-buffered NaCl produced no immediate effects on the ability of the cells to return to discoid shape with reoxygenation. When deoxygenation was continued for several hours, however, the percentage of ISC gradually increased, even in the presence of 1 mM EGTA. It reached approximately 20% after 4–4.5 hr of continued deoxygenation after sucrose was added to produce an osmotic strength of 500 mosmole/kg (Fig. 1). Control suspensions, maintained at 290 mosmole/kg throughout the period of deoxygenation, did develop a small percentage of ISC, but the appearance of those ISC was slower than in the hypertonic medium. In addition to this moderate increase of ISC with prolonged deoxygenation in hypertonic medium, a varying proportion of cells was observed.

Morphological Evaluation

The morphology of glutaraldehyde-fixed cells was assessed by phase contrast microscopy. Selected samples were also processed further by conventional techniques for scanning electron microscopy.7 Additional samples were processed and embedded in Epon for transmission electron microscopy.1

RESULTS

Retention of Sickled Shape by Reoxygenated Cells

Simple hyperosmotic dehydration of non-ISC sickled in isotonic-buffered NaCl produced no immediate effects on the ability of the cells to return to discoid shape with reoxygenation. When deoxygenation was continued for several hours, however, the percentage of ISC gradually increased, even in the presence of 1 mM EGTA. It reached approximately 20% after 4–4.5 hr of continued deoxygenation after sucrose was added to produce an osmotic strength of 500 mosmole/kg (Fig. 1). Control suspensions, maintained at 290 mosmole/kg throughout the period of deoxygenation, did develop a small percentage of ISC, but the appearance of those ISC was slower than in the hypertonic medium. In addition to this moderate increase of ISC with prolonged deoxygenation in hypertonic medium, a varying proportion of cells was observed.

In various experiments involving dehydration of sickled cells, the cells were deoxygenated for 90–120 min before dehydration, and cell samples were then fixed for verification of sickling. At various stages of the experiments, a measure of cell dehydration was obtained by determination of MCHC as already described. The monovalent cation content of cells was determined by flame photometry. For these measurements cells were washed 3 times in isotonic Tris-buffered magnesium chloride solution (Tris-MgCl₂, 10 mM Tris-HCl, pH 7.4) and were then lysed in LiCl solution (15 meq/liter, Instrumentation Laboratories). The quantity of cations per volume of cells was calculated using the hematocrit of the washed cell samples.

<table>
<thead>
<tr>
<th>Non-disc Cells - %</th>
<th>sucrrose added</th>
<th>ISC + spiked cells</th>
<th>ISC</th>
<th>spiked cells</th>
<th>total sickled cells</th>
<th>290 mosm control</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>10</td>
<td>20</td>
<td>30</td>
<td>40</td>
<td>50</td>
<td>60</td>
</tr>
</tbody>
</table>

Fig. 1. Retention of sickled shape in hypertonic medium. Discoid sickle cells were deoxygenated in isotonic buffered medium containing 1 mM EGTA. After 2 hr, when the cells were well sickled, a deoxygenated sucrose solution was added to increase the osmotic strength to 500 mosmole/kg. After continued deoxygenation for the indicated periods, samples were removed and reoxygenated. The percentages of smooth contoured ISC and spiked sickled cells are plotted singly and as a sum. Note that between 5 and 8 hr, the sum of ISC and spiked cells remained constant, and the spiked cells increased at the expense of ISC.
that retained the spiky morphology of deoxygenated non-ISC after reoxygenation. In the experiment summarized in Fig. 1, the percentage of such cells was 11% 3 hr after sucrose addition and rose to 24% in 3 more hr. This increase was at the expense of an equal percentage of ISC. The cell shape was clearly distinguishable from the rounded profile of ISC. This retention of sickled shape was reminiscent of the appearance of "fixed" sickle cells obtained with Ca and A23187, which we and others had previously described. After reoxygenation, cell samples from one of these experiments were centrifuged on a 500-mosmole/kg Stractan gradient to determine the relative density of cells of sickled morphology. The proportion of spiked sickled cells increased markedly in the high density portion of the gradient; in fact, most of them went all the way through the high-density cushion. This suggested that the spiked sickled cells were derived from particularly dehydrated cells.

To understand the association between the production of "fixed" sickle cells and cellular dehydration, we varied the amount of sucrose added after deoxygenation. A greater percentage of "fixed" sickle cells was noted at 600 mosmole/kg than at 500 mosmole/kg osmotic strength (Table 1). No substantial increase of these cells occurred at 700 mosmole/kg.

Since Glader had suggested that potassium loss, as well as reduction in cell water, was required for irreversible sickling, we used Nystatin to vary the cation content of sickled cells. Cells were treated with Nystatin in high-K or low-K medium, so that the intracellular K concentration was maintained or reduced. Sucrose was also added to reduce the total water content. The presence of Nystatin permitted net efflux of Na and K with overall reduction of ion content. Under conditions where a substantial loss of cell water and cations occurred, the effect on sickle cell morphology was striking. Reoxygenation had virtually no effect on cell shape. The sickled cells with spiked extensions retained their spiculated form, as though they had been fixed in glutaraldehyde (Fig. 2A). When Nystatin and sucrose were added under conditions that did not produce such severe dehydration (Table 2), fixation of the sickled form was diminished or did not occur, and the cells resumed a smooth discoid morphology after reoxygenation. With intermediate degrees of dehydration, the cells unsickled, but did so slowly, requiring 10–30 min to return to discoid shape. The composition of monovalent cations did not appear to affect the results; only the ion and water content of the cells was influential in the fixation of sickled form. Thus, "fixed" sickle cells were obtained in both high and low-K medium with sufficient dehydration.

Table 1. Effect of Medium Osmolality on Stabilization of Sickled Shape

<table>
<thead>
<tr>
<th>Medium Osmolality (mosmole/kg)</th>
<th>% Sickled Cells – O2</th>
<th>% Sickled Cells – N2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>290</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>500</td>
<td>0.08</td>
<td>0.39</td>
</tr>
<tr>
<td>600</td>
<td>0.38</td>
<td>0.64</td>
</tr>
<tr>
<td>Experiment B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>290</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>500</td>
<td>0.02</td>
<td>0.10</td>
</tr>
<tr>
<td>700</td>
<td>0.36</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Discoid sickle cells were deoxygenated in isotonic buffered medium containing 1 mM EGTA. After the cells were sickled, sucrose was added anaerobically to give the indicated osmolalities. At the designated times after raising the osmolality, samples were reoxygenated, and the percentage of sickle cells compared with that in the deoxygenated samples.

The Nystatin experiments suggested that our earlier observations of "fixed" sickle cells following exposure to Ca and A23187 could have been the result of K loss and dehydration. Thus, we extended our earlier experiments involving the addition of A23187 to sickled cells in Ca-containing solutions to test this possibility. To block K loss and cell dehydration, some experiments were conducted in high-K medium. Also, the ionophore concentration in the present experiments was reduced from 20 μM to 2 and 5 μM. When ionophore was added to sickled cells in the low-K medium, fixation of the sickled form was obtained in the presence of 10 and 100 μM Ca. The spiculated sickled morphology was retained even after 90-min reoxygenation. In contrast, when the ionophore was added to sickled cells in high-K medium, no "fixed" sickle cells were observed. As the data in Table 3 show, this difference in the cell morphology was accompanied by a corresponding difference in cell hydration. The cells in low-K medium lost water and increased their MCHC, whereas the cells in high-K medium did not lose potassium and maintained a low MCHC. It should be noted that the cells in high-K medium showed a normal morphological response to...
Fig. 2. Scanning electron micrographs of Nystatin- and Ca/A23187-treated sickle cells. (A) Sickled cells were treated with Nystatin in high-K medium at 500 mosmole/kg and were then reoxygenated for 30 min. Note retention of highly spiculated sickled morphology. (B) Spiculated cells as shown in (A) were washed in isotonic medium with concomitant reduction of MCHC. Note reversion to discoid morphology. (C) Sickled cells in low-K, isotonic medium containing 10 μM CaCl₂ were treated with 2 μM A23187 and then reoxygenated for 30 min. Note spiculated morphology. (D) Spiculated cells as shown in (C) were washed in 155 mosmole/kg buffered medium. Note reversion to discoid and echinocytic morphology.

Ca once they were reoxygenated. That is, the cells became echinocytic (except those in the presence of 2 μM A23187 and 10 μM Ca, where many cells resumed a smooth discoid morphology).

Reversal of “Fixed” Sickle Cells

Whereas “fixed” sickles generated by the addition of Nystatin and sucrose maintained a spiculated sickled morphology even during overnight storage at 0°C, they were found to unsickle immediately when washed in isotonic medium (Fig. 2B). Both isotonic phosphate-buffered saline and Tris-MgCl₂ caused this reversal, whether or not Nystatin had been previously removed by washing in warm medium.

It was also possible to reverse the “fixed” sickle cells generated in the presence of A23187 and Ca (Fig. 2C

<table>
<thead>
<tr>
<th>Table 2. Retention of Sickled Shape After Treatment With Nystatin and Sucrose</th>
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<tbody>
<tr>
<td><strong>% Sickled Cells – O₂</strong></td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>High-Na medium</td>
</tr>
<tr>
<td>290 mosmole/kg</td>
</tr>
<tr>
<td>400 mosmole/kg</td>
</tr>
<tr>
<td>500 mosmole/kg</td>
</tr>
<tr>
<td>High-K medium</td>
</tr>
<tr>
<td>290 mosmole/kg</td>
</tr>
<tr>
<td>400 mosmole/kg</td>
</tr>
<tr>
<td>500 mosmole/kg</td>
</tr>
</tbody>
</table>

Discoid sickle cells were deoxygenated in high-Na or high-K medium. After they had sickled, Nystatin and sucrose were added to give the indicated osmolalities. Cells were fixed in glutaraldehyde both anaerobically and after reoxygenation for determining the retention of sickled shape.

*meq/liter cells.
Deoxygenation of Dehydrated Cells

During the early experiments designed to elucidate the effects of cell dehydration on irreversible sickling, we found that cells that were dehydrated before deoxygenation did not appear to sickle. This effect was independent of the means of dehydration, since cells suspended in solutions made hypertonic with either NaCl or sucrose failed to develop the characteristic sickled shape. Cells dehydrated with Nystatin and sucrose to produce an MCHC of 46 g/dl in isotonic medium also failed to develop the typical sickled morphology when deoxygenated. Instead, they became roughened and lumpy, while retaining an overall discoid form (Fig. 4A). A few very fine, often branched, extensions were observed around the cell periphery. These could be seen by scanning electron microscopy, but were too fine to be readily visible in the phase-contrast microscope. When these Nystatin-dehydrated cells were deoxygenated in hypotonic medium, they did develop a spiculated morphology more typical of sickled cells (Fig. 4B). This sickling reversed readily upon reoxygenation.

Prompted by these observations, we deoxygenated isolated subpopulations of ISC in isotonic and hypotonic media. When deoxygenated at 290 mosmole/kg, ISC did not change their overall shape, but the cell surface assumed a roughened appearance (Fig. 5A) as others have already noted.4 When the same ISC were dehydrated at 120 mosmole/kg, however, a large proportion of the cells developed spiculated extensions like those of deoxygenated non-ISC (Fig. 5B). These cells resumed their original ISC morphology with reoxygenation.

Microscopic Organization of Hemoglobin S

Transmission electron microscopy of “fixed” sickle cells revealed no hemoglobin S polymers, whether the “fixation” was achieved with Nystatin or Ca and A23187. These observations are consistent with the results of Eaton et al.13 concerning the transient retention of sickle shape at higher concentrations of Ca and ionophore. There was no apparent change in the interior of “fixed” sickle cells when washing had caused them to lose their sickled morphology. In contrast to

![Graph showing the dependence of Nystatin “fixed” sickling on monovalent cation concentration.](image-url)

**Deoxygenation of Dehydrated Cells**

Complete reversal occurred with reduction of the medium osmolality to 155 mosmole/kg. Partial reversal occurred with washing in isotonic phosphate-buffered saline containing 1 mM EDTA and 0.1% bovine serum albumin. When Ca was substituted in the wash media, the same reversal behavior was seen, thus excluding loss of Ca as a mechanism for reversal. Washing the Ca “fixed” sickle cells resulted in reduction of the MCHC, even in isotonic medium. Thus, the reversal process was associated, as in the Nystatin experiments, with an increase in cell water content. Some of the ionophore-treated cells showed a morphology similar to that of native ISC after washing and reversal of the “fixation” phenomenon (Fig. 2D). Such ISC-like cells were not produced in high-K medium.

**Table 3. Retention of Sickled Shape After Treatment With Ca and A23187**

<table>
<thead>
<tr>
<th>% Sickled Cells – O₂</th>
<th>MCHC (g/100 ml)</th>
<th>Intracellular Cations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Na⁺</td>
</tr>
<tr>
<td>High-K, 2 μM A23187</td>
<td>0</td>
<td>34.2</td>
</tr>
<tr>
<td>High-K, 5 μM A23187</td>
<td>0</td>
<td>34.5</td>
</tr>
<tr>
<td>Low-K, 2 μM A23187</td>
<td>0.56</td>
<td>55.2</td>
</tr>
<tr>
<td>Low-K, 5 μM A23187</td>
<td>0.34</td>
<td>54.8</td>
</tr>
</tbody>
</table>

Discoid sickle cells were deoxygenated in high-K and low-K media containing 10 μM added CaCl₂. After the cells had sickled, A23187 was added to the indicated final concentrations. Deoxygenated and reoxygenated samples were fixed in glutaraldehyde for counting.

*meq/liter cells.
the homogeneous appearance of the cell interior of reoxygenated “fixed” sickle cells, deoxygenated ISC showed variable formation of hemoglobin S polymer. In both isotonic and 120 mosmole/kg medium, some cells were devoid of filamentous structures, whereas others were filled with obvious hemoglobin S rods. Thus, despite their distinct difference in morphology, isotonic and hypotonic deoxygenated ISC were indistinguishable by transmission electron microscopy.

DISCUSSION

The ISC has been discussed by many authors as an example of a cell in which membrane “rigidity” is responsible for an abnormal shape and reduced whole cell deformability. Thus, perhaps as a result of Ca accumulation, it was thought that the ISC membrane was effectively “fixed” into the deformed conformation of the sickled cell. In addition, this conformation was retained by membranes after hemolysis or even by the spectrin actin “cytoskeleton” of the cell. Furthermore, the reduced deformability of ISC and their failure to demonstrate morphological sickling upon deoxygenation were explained in terms of changes in the viscoelastic properties of the membrane, expressed as a loss of whole cell deformability.

In contrast to this view, the present experiments suggest that cell water content may be more important than intrinsic membrane properties in limiting the morphological response of sickle cells to changes in oxygen tension. Cells that lose excessive water while sickled retain a spiculated sickled morphology after reoxygenation. Cells that are dehydrated before deoxygenation lose their capacity to form the extended spicules that are characteristic of sickled cells with normal water content. Rehydration restores the characteristic sickling and unsickling response of dehydrated cells, showing that loss of membrane deformability is not the mechanism by which cell shape
changes were inhibited. These observations are summarized in schematic form in Fig. 6.

Once it became clear the phenomenon of “fixed” sickling was dependent on severe ion and water loss during sickling, we postulated a simple mechanism based on the absence of sufficient water to redissolve the hemoglobin S polymers. Thus, it was surprising to find that no hemoglobin fibers could be detected in transmission electron micrographs of “fixed” sickle cells. Although Eaton et al. had made the same observation in their sickling experiments at higher Ca/A23187 concentrations, we had thought that the absence of hemoglobin fibers might have been related to the transience of the shape stabilization in those experiments. Since no fibers were found even in our experiments, in which the sickled cells retained their spiked shape for several hours, the retention of spiculated morphology cannot be explained by the presence of large bundles of hemoglobin polymer. At present, there is no obvious explanation for the stability of the “fixed” sickle morphology, unless the hemoglobin inside is so concentrated that it behaves as a semisolid substance. Certainly, the severity of dehydration that is necessary for “fixed” sickling suggests that the cell interior is not fluid. High viscosity also is a reasonable explanation for the failure of dehydrated discoid sickle cells and ISC to elaborate long spicules upon deoxygenation. Those cells were not as severely dehydrated, however, and a limited morphological response could be seen.

These experiments extend our understanding of the previously studied effects of Ca and A23187 on the morphology of sickle cells. Those experiments were puzzling, in that the “fixed” sickle morphology was clearly different from that of native ISC. Thus, the relationship between the phenomenon of Ca stabilization of sickled cells and irreversible sickling was not evident. Now it is apparent that the spiculated morphology of Ca “fixed” sickle cells is the result of Ca-mediated ion and water loss—the Gardos effect. Reversal of Ca “fixed” sickle cells did reveal, moreover, the presence of a few cells with a morphology similar to that of native ISC. The fact that such cells were not found when ion and water loss was blocked by high-K concentrations outside the cell suggests that Ca entry together with total ion loss were involved in the shape change. The absence of ISC-like cells after hypotonic reversal of Nystatin “fixed” sickle cells would also support a partial role for Ca in the generation of ISC morphology. However, it should be kept in mind that ISC were observed with simple sucrose dehydration in the presence of EGTA. All these observations are difficult to reconcile with a direct effect of Ca on irreversible sickling. They suggest that ion and water loss cooperate with at least one other factor in the production of ISC. This other factor may be produced either by Ca accumulation or by other, as yet undefined, time-dependent processes. Palek has recently reviewed the various data on both sides of this controversial question.

The gradual appearance of “fixed” sickle cells in hypertonic medium that did not contain Nystatin supports the idea that this phenomenon requires a reduction in total ion content as well as simple water loss. Incubation of sickled cells in medium made hypertonic with sucrose would have increased the potassium gradient across the membrane while slightly reducing the sodium gradient. Thus, over a period of time, the cells would tend to lose excess K and reduce their total ion content.

The present experiments suggest a revised concept of the ISC membrane as a more deformable entity than previously thought. This idea is also consistent with our recent studies of ISC deformability at various osmolalities. In those experiments we found that suspension of ISC in hypotonic medium restored deformability to the cells. This also suggests that the membrane does not severely restrict the alteration of cell shape in response to stress, whether imposed by growth of deoxyhemoglobin polymers or by application of external shear stress.

An objection to this idea of a flexible ISC membrane might be raised on the basis that ISC contain excess amounts of Ca and that elevated Ca is thought to induce “rigidification” of red cell membranes. However, experiments in our laboratory have shown that reduced deformability in normal cells loaded at low Ca concentrations (10–100 μM) can be explained totally on the basis of the Gardos effect. Reduction of the medium osmolality of such Ca-loaded undeformable cells completely restores their deformability.
If ISC membranes are not frozen into their abnormal shape by a loss of membrane deformability, how can we explain the unusual shape of ISC ghosts and the memory of that shape by the putative cytoskeleton? We suggest that in this regard, ISC membranes may behave like normal discocyte membranes, which also retain a discocytic shape after hemolysis or Triton extraction. The difference in shape may be the result of plastic flow of the membrane while the cells were in sickled configuration. This morphological accommodation could be mediated by a spatial reorganization of noncovalent bonding involving those membrane-associated proteins that are thought to provide a skeletal network. Such a reorganization could be purely geometric, without necessarily changing the viscoelastic properties of the membrane in an appreciable manner. Plastic flow of normal membranes as a result of prolonged stress imposed by a micropipet has been observed and provides an analogy for such a process.

The present studies do not rule out the existence of altered membrane properties in ISC. However, they do suggest that other cellular properties, especially reduced cell water content, should be given more consideration in efforts to understand the unusual behavior of these cells.

REFERENCES

10. Reed, PW: Calcium-dependent potassium efflux from rat erythrocytes incubated with the antibiotic A23187. Fed Proc 32:635, 1973
Influence of red cell water content on the morphology of sickling

MR Clark, JC Guatelli, N Mohandas and SB Shohet