Permeability Characteristics of Deoxygenated Sickle Cells

By Margaret R. Clark and Mary E. Rossi

This study investigated the effect of acute deoxygenation on membrane permeability characteristics of sickle cells. Measured fluxes of Na\(^+\) and K\(^+\) in ouabain-inhibited cells, of chloride and sulfate exchange in 4,4'-diisothiocyanostilbene-2,2'-disulfonate (DIDS)-inhibited and untreated cells, and of erythritol, mannitol, and arabinose in cytoclasalin B-inhibited cells indicated that a deoxygenation-induced permeability change occurred in sickle cells only for cations and chloride. Monovalent cation permeabilities increased fivefold, and chloride influx into DIDS treated cells was enhanced nearly threefold on sickle cell deoxygenation. In contrast, no detectable increase in permeability to the other solutes was found. To gain perspective on these findings, similar measurements were performed in normal sickle cells treated with diamide, an agent shown by others to induce a coupled increase in membrane permeability and phospholipid translocation, reminiscent of deoxygenation-induced changes in sickle cells. Although the increased cation permeability was no greater than that in sickled cells, treatment with 2 mmol/L diamide also produced a twofold increase in the first order rate constants for sulfate exchange and mannitol efflux, indicating a relatively nonselective permeability increase that permitted flux of larger solutes than in the case of deoxygenated sickle cells. These results suggest that the deoxygenation of sickle cells induces a permeability increase that is relatively insensitive to charge, but is restrictive with respect to solute size.

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THE PRIMARY DEFECT in sickle cell disease is the abnormal structure of the hemoglobin S (Hbs) molecule, whose polymerization at low oxygen tension causes the major clinical manifestation, vasoocclusion. Nevertheless, it is thought that some of the factors that initiate vasoocclusive events and promote rapid destruction of sickle cells may be related to effects of Hbs on red blood cell (RBC) membrane structure and function. 1-3 Most of the investigation of membrane abnormalities in sickle cells has focused on long-term, cumulative damage such as that seen in irreversibly sickled cells (ISC). However, for a complete understanding of the nature and pathophysiologic implications of membrane damage in sickle cells, it is important to define each stage of the process. From this perspective, we have begun to investigate the nature of the early changes in RBC membrane permeability that are associated with sickling. More than 30 years ago, Tosteson et al reported that acute deoxygenation of sickle cells produced an increase in membrane permeability to alkali cations. 4,5 Subsequent work showed that in addition to monovalent cations, Ca\(^{2+}\) also could enter sickle cells at an increased rate when they were deoxygenated. 6 The relationship of these observations to the extreme dehydration of a subpopulation of sickle cells has been debated, 7,8,9 but as yet there is insufficient information about the nature of the permeability change to determine how it fits into the development of sickle cell membrane injury. We previously postulated that the extreme distortion of the membrane at the tips of spicules on sickled cells might result in a breach in the membrane permeability barrier. In accord with this hypothesis, we found that the magnitude of the sickling-induced leak for Na\(^+\) and K\(^+\) varied in parallel with the extent of cell distortion.10 One might expect that such a mechanically induced leak would show relatively little selectivity for different types of solutes. Such a lack of specificity has been observed by Deuticke et al11 and Schwister and Deuticke12 for permeability defects induced by treating normal cells with oxidizing agents or electric discharge. Interestingly, these investigators also found that the permeability increase was accompanied by an increase in the rate of translocation of phospholipids across the membrane bilayer, qualitatively similar to the increased transbilayer movement that is induced by deoxygenation of sickle cells.14 Thus, we hypothesized that if the permeability change in acutely deoxygenated sickle cells was the result of mechanical disruption of bilayer structure, the cells would exhibit a nonselective increase in membrane permeability to a variety of solutes, similar to that induced by diamide treatment of normal RBCs.

An alternative explanation for increased monovalent cation fluxes in sickled cells could be a sickling-induced activation of specific cation transport pathways. Studies have been performed by others in an effort to detect sickling-associated abnormalities in Na\(^+\)/K\(^+\) cotransport, the volume-sensitive KCl transport system, or Na\(^+\)/K\(^+\) countertransport, but no effect of deoxygenation on flux through these routes in sickle cells was found. However, Joiner and Dew did find that an inhibitor of anion exchange, 4,4'-diisothiocyanostilbene-2,2'-disulfonate (DIDS) blocked the increase in cation fluxes in deoxygenated sickle cells, and they originally suggested that the increased cation fluxes might possibly be mediated by an altered form of the anion transport protein.17

In an effort to test whether the sickling-induced membrane permeability defect represents a nonselective breakdown of the membrane barrier, we have measured the effect of deoxygenation on the permeability of sickle cells to a variety of solutes, including the sulfate and chloride anions and three neutral solutes of different sizes. Direct comparison of permeability changes in sickled cells with the observations of Deuticke et al on diamide-treated cells seemed inappropriate, because those experiments were performed at levels of oxidant treatment that gave larger changes in permeability and lipid translocation than are seen in deoxygenated sickle cells.
cells. Therefore, we performed additional flux measurements, using normal cells treated with lower concentrations of diamide to provide a model of nonselective permeability increase for comparison with the sickle cells.

**MATERIALS AND METHODS**

**Blood samples.** Blood samples were obtained from sickle cell patients at the Northern California Comprehensive Sickle Cell Center, San Francisco General Hospital, after obtaining informed consent as approved by the Committee on Human Research of the University of California at San Francisco. All blood samples were from patients who were homozygous for Hb S, as determined by thin-layer isoelectric focusing and citrate agar electrophoresis. The α-globin genotype was not determined.

Blood samples from patients and control subjects were drawn into acid citrate dextrose (ACD; Becton Dickinson, Rutherford, NJ) or CPD-A (Fenwal Laboratories, Deerfield, IL) anticoagulant and were normally used the day after they were obtained, although on occasion they were used after storage for 3 to 5 days. To obtain a more homogeneous population of sickle cells, arabinogalactan gradients were used to isolate an intermediate density cell population from which the youngest reticulocytes and most of the ISC had been removed. The cells collected usually had densities of 1.077 to 1.103 g/mL, but occasionally a narrower density range was selected when a sufficient number of cells for the experiment could be isolated.

**Diamide treatment of normal cells.** At the 5 mmol/L concentration used by Deuticke et al,16 diamide produced much greater increases in the rates of phospholipid translocation and cation flux than those induced by deoxygenation of sickle cells.18,20 Thus, in the current studies, a lower concentration was used, to give a more meaningful comparison of the effects of these two manipulations. A concentration of 2 mmol/L diamide was chosen, because treatment of normal cells at this level gave an increase in K+ efflux comparable with that of deoxygenated sickle cells. The rate of lipid translocation at this level has been shown to be approximately three times that in deoxygenated sickle cells.12,13 For diamide treatment, the cells were incubated for 60 minutes at 37°C, in 90 mmol/L KCl, 45 mmol/L NaCl, 44 mmol/L sucrose, and 10 mmol/L Na phosphate, pH 8.0. They were then washed and prepared as described below for the flux experiments.

**General method for flux experiments.** Before initiation of all efflux experiments involving sickle cells, paired samples of preloaded cells were placed on ice, and one of each pair was deoxygenated by equilibration of the cell suspension with humidified nitrogen gas. Because at 0°C sickling does not occur, and baseline permeability is low, this prevented a large contribution to the measured fluxes during the relatively slow deoxygenation process. Once the samples were deoxygenated, they were sealed, and all samples were rapidly brought to the temperature at which the flux was to be measured, usually 37°C. For influx experiments, samples were first deoxygenated at room temperature. Then they were warmed to 37°C for at least 5 minutes, the isotope was added (under a nitrogen atmosphere for the deoxygenated samples), and the zero time sample removed immediately after mixing. For all flux experiments, samples were removed at intervals depending on the rate of the flux process to provide at least four sequential determinations. Deoxygenated samples were removed under nitrogen, and the incubation vessels resealed after each sampling. Experiments using diamide-treated normal cells were performed without prior deoxygenation.

For efflux measurements, suspension samples were centrifuged, and the supernatant analyzed for the effluxing solute. For influx measurements, cells were separated from the medium containing the test solute and assayed for its accumulation. In all but the sulfate flux experiments, measurements of the hematocrit and Hb concentration (measured as the cyanomethemoglobin complex) on an initial cell sample were used in conjunction with measurements of the Hb concentration in the incubation mixture to determine the hematocrit of the incubation mixture, thereby permitting expression of measured fluxes on the basis of original cell volume. Efflux measurements were corrected for hemolysis using measured values of the Hb concentration in the supernatant. In the sulfate flux experiments, the sulfate in the medium interfered with measurement of Hb as the cyanomethemoglobin complex, and the absorbance of the supernatant at the Soret band (415 nm) was used to determine the hemolysis correction. The hematocrit during incubation was determined from a RBC count using a Coulter Model B cell counter (Coulter Electronics, Hialeah, FL) and the RBC count and hematocrit of the initial washed cell suspension.

**Sodium and potassium fluxes.** The net fluxes of Na⁺ and K⁺ were determined during incubation of the cells for 4.5 hours, 37°C, at approximately 4% hematocrit in isotonic NaCl buffered with 10 mmol/L Na phosphate, pH 7.4. Cell samples were washed three times in ice-cold, isotonic Tris buffered (10 mmol/L, pH 7.4) MgCl₂, and then assayed for Na⁺ and K⁺ content by flame photometry. In addition, samples of the suspending medium were assayed for K⁺ to provide an alternate measurement of K⁺ efflux. To determine the effect of DIDS on the cation fluxes, paired samples were incubated with and without the addition of 100 μmol/L DIDS (Sigma Chemicals, St Louis, MO). In all cation flux experiments presented here, the DIDS was present during the flux measurements. Another approach was tried in which the cells were pretreated with DIDS and then washed before proceeding with the flux measurements, but this method caused increased hemolysis in the DIDS-treated cells during subsequent incubation and was therefore abandoned.

**Sulfate fluxes.** Sulfate flux was determined as the efflux of "SO₄²⁻ (New England Nuclear, Natick, MA) from cells that had been previously loaded with the radiolabeled anion. The experiments were performed after the cells had been incubated at 37°C for at least 2 hours in medium containing 20 mmol/L sulfate, which was found to be adequate for sulfate equilibration in the absence of DIDS; thus, the subsequently measured sulfate fluxes in the same medium (0.13 mol/L sucrose, 40 mmol/L NaCl, 20 mmol/L Na₂SO₄, and then 18 mmol/L Tris-HCl, pH 7.4 at 37°C) represented the rates of sulfate exchange, rather than net flux. The isotope concentration was adjusted to give a specific activity of approximately 0.5 mCi/mmol/L of sulfate. For the efflux experiments, the isotope was added during the last 2 hours of a 4-hour preincubation. Before measuring efflux of the label from preloaded cells, half of each cell sample was treated with DIDS (50 or 100 μmol/L) to inhibit carrier-mediated anion exchange, and the cells were then washed in ice-cold incubation medium to remove extracellular isotope. The intracellular sulfate concentration at the beginning of the flux experiments was determined from the radioactivity of the washed, loaded cells, together with the specific activity of the loading medium. Subsequent loss of labeled sulfate from the cells was then quantitated from the increase of isotope counts in the supernatant of centrifuged samples and the hematocrit of the incubation suspension. Sulfate efflux was measured over a 1-hour period of incubation at 37°C.

Because the efflux measurements represented the redistribution of the isotopically labeled anion in cell suspensions that had already been equilibrated with sulfate, the rates were expected to follow first-order kinetics. Therefore, the data were analyzed by plotting the natural log of the ratio of intracellular sulfate concentration to the initial concentration versus time. This procedure compensates for the variation in intracellular sulfate concentration after cell loading. In particular, diamide-treated cells never retained as much sulfate as...
untreated cells while they were being washed after the loading procedure.

In addition to the efflux experiments, we also performed a series of experiments in which we determined the rate of influx of $^{35}\text{SO}_4^{2-}$ into cells preequilibrated in nonradioisotopic sulfate solutions. The precision in measuring uptake of the slowly equilibrating sulfate into DIDS-treated cells is inherently much lower than that of the efflux experiments, so a modest increase in influx would not have been detected. This approach was therefore abandoned.

**Chloride influxes.** Because chloride fluxes in cells with a functional anion transport system are too rapid to be measured at 37°C, we performed influx experiments for DIDS-inhibited cells. Washed sickle cells were pretreated with 100 μmol/L DIDS in BSKG (isotonic phosphate-buffered saline containing 10 mmol/L sodium phosphate, pH 7.4, 5 mmol/L potassium chloride, and 11 mmol/L glucose) for 30 minutes at room temperature, and one of each paired sample was deoxygenated. The flux experiment was initiated after placing the samples in a 37°C water bath for a 5-minute equilibration period. Then radiolabeled chloride was added, and four consecutive samples were taken, to provide 0-, 5-, 10-, and 20-minute values for chloride uptake. The duplicate aliquots were immediately introduced into ice-cold incubation medium layered over dibutylphthalate and centrifuged to separate the cells from the extracellular isotope. The cell pellet was then lysed and a sample taken for Hb determination. Aliquots of the remaining lysate were then treated with 10% trichloroacetic acid to precipitate protein, centrifuged, and aliquots of supernatant counted to determine the cell content of labeled chloride.

**Neutral species effluxes.** Three solutes were used to determine the effect of deoxygenation on permeability to neutral species. They were 14C-labeled erythritol (Amersham Corp, Arlington Heights, IL), mannitol, and arabinose (ICN Biomedicals, Inc, Costa Mesa, CA). For these experiments, cells were preloaded under room air by incubation in 4 mmol/L solute (0.5 μCi/mL) at 37°C for 2 hours, in medium containing 100 mmol/L KCl, 36 mmol/L NaCl, 6.5 mmol/L sodium phosphate, pH 7.2, and 44 mmol/L sucrose, as described by Deuticke et al. The cells were then washed and resuspended in the same medium, to which cytochalasin B (10 μmol/L) was added to inhibit facilitated diffusion. Sickle cell samples were maintained on ice during the deoxygenation period, then the samples were warmed to 37°C, and efflux rates were determined by measuring the accumulation of radioactivity in the supernatant of centrifuged samples. In experiments using erythritol and mannitol, rate constants were calculated from the apparent first order efflux over the first 40 to 60 minutes. For arabinose efflux in the diamide experiments, the efflux during the first 20-minute time interval did not fit into a first order process with subsequent points. Therefore, first order rate constants for all arabinose experiments were calculated for the data between 20 and 120 minutes, which did give a good fit to first order kinetics. To be sure that the effect of diamide on mannitol efflux from normal cells was not due to the presence of contaminating white blood cells, one experiment was performed in which leukocytes and platelets were rigorously removed by filtration of the whole blood through cellulose.

**Analysis of results.** A Statview program (Brain Power, Inc, Calabasas, CA) on a Macintosh II computer (Apple Computers, Inc, Cupertino, CA) was used to calculate the mean and standard errors for replicated experiments, and to perform paired and unpaired t-tests as appropriate. A Cricket Graph program (Cricket Software, Malvern, PA) was used to obtain first order rate constants for efflux measurements by performing linear regression of the log of the intracellular concentration of the effluxing solute versus time.

**RESULTS**

Our initial experiments involved the measurement of $^{35}\text{SO}_4^{2-}$ efflux from sickle cells in the deoxygenated versus oxygenated state. The first few experiments showed a substantial deoxygenation-induced increase in sulfate efflux from DIDS-treated cells, even after correction of the total supernatant radioactivity for the slight amount of hemolysis that occurred. In marked contrast to these initial results, subsequent experiments using a new lot of DIDS failed to show any deoxygenation-induced increase in sulfate efflux from DIDS-treated or untreated cells (Figs 1 and 2, Table 1). In the analysis of these experiments, the total sulfate efflux was corrected for hemolysis, which ranged from 0.4% to 3% total, and 0.2% to 1.5% net increase during the efflux experiments.

In contrast to the lack of effect of deoxygenation on sulfate efflux from DIDS-treated sickle cells, treatment of normal cells with 2 mmol/L diamide caused a twofold increase in DIDS-resistant sulfate efflux (Fig 2B, Table 1). No effect of...
diamide could be detected in cells that were not treated with DIDS (Fig 1B).

Although deoxygenation had no effect on efflux of sulfate from sickle cells, it produced a consistent increase in the rate of $^{36}$Cl accumulation by sickle cells that had been treated with DIDS to suppress active anion exchange (Fig 3). The magnitude of the effect varied from experiment to experiment, giving an average increase of between twofold and threefold in the rate of chloride uptake over a 20-minute period ($0.005 < P < 0.01$). In only one of seven experiments was there no significant difference in chloride uptake by deoxygenated as compared with oxygenated cells.

The effect of DIDS on the deoxygenation-induced cation leak was also studied. As reported by Joiner, $^{24}$ DIDS reduced both $K^+$ efflux and $Na^+$ influx in deoxygenated sickle cells to approximately half the values in the absence of DIDS (Fig 4). The difference in fluxes between oxygenated and deoxygenated cells was decreased by an even larger amount, because DIDS treatment actually increased the $Na^+$ and $K^+$ fluxes in oxygenated sickle cells. It should be noted that early experiments, conducted at the same time as the preliminary sulfate efflux experiments that had suggested a deoxygenation-dependent sulfate leak, showed a much smaller effect of DIDS on the cation fluxes as well. Presumably the DIDS used in these early experiments was not fully active.

Cation fluxes were also measured for normal cells treated with 2 mmol/L diamide. Diamide treatment induced a large increase in cation permeability, comparable with the permeability increase seen on deoxygenation of sickle cells (Fig 4). However, treatment with DIDS did not reduce the enhancement of $K^+$ and $Na^+$ fluxes. In fact, when the effect of DIDS on fluxes from diamide-treated and untreated cells was analyzed using a $t$-test for paired samples (with and without DIDS), only the $Na^+$ influx into diamide-treated cells was significantly different from the flux in DIDS-free cells, and it was increased ($0.01 < P < 0.025$). The $P$ values for the effect of

| Table 1. Summary of Rate Constants for DIDS-Resistant Exchange of Sulfate in Sickle and Diamide-Treated Normal RBCs |
|-------------|-------------|-------------|-------------|-------------|
| Sickle Cells | Oxy         | Deoxy       |
| Normal Cells | Control     | 2 mmol/L Diamide |
| Rate constant--h$^{-1}$ | 0.034 (0.013) | 0.035 (0.010) | 0.042 (0.006) | 0.089 (0.013)* |

First order rate constants were calculated by linear regression analysis of the data from four experiments with each type of cells. All cells were treated with 50 μmol/L DIDS.

$^*$ $0.005 < P < 0.01$ for diamide-treated as compared with control normal cells.
To determine whether deoxygenation of sickle cells made them more permeable to uncharged solutes, we used three different 14C-labeled probes, erythritol, mannitol, and arabinose. The rates of flux of these molecules across the RBC membrane vary over a broad range, reflecting their different sizes. As shown in Table 2, the first order rate constants for all three species were unaffected by deoxygenation of sickle cells. Rates of mannitol efflux from both deoxygenated and oxygenated sickle cells were higher than those observed for normal control cells, but the number of experiments is too small to determine whether this difference was consistent.

When the effect of diamide on the permeability of normal cells was examined, we found that treatment with 2 mmol/L diamide did produce an approximately twofold increase in the efflux of mannitol (.025 < P ≤ .005). There was no detectable alteration in the permeability to erythritol or arabinose induced by this treatment (Table 3). Because Deuticke et al had found a greater permeability increase for erythritol than for mannitol with diamide treatment at 5 mmol/L concentration, we compared erythritol efflux from cells treated with 0, 2, and 5 mmol/L diamide, but found no difference (data not shown). We also considered the possibility that the increased mannitol efflux from diamide-treated samples might be due to the presence of contaminating leukocytes. Cells were prepared with and without prefiltration through cellulosene to remove white blood cells and platelets, and were then treated with 2 mmol/L diamide. There was no difference in the loading of mannitol or its subsequent efflux (data not shown). Because of the very low permeability of the membrane to mannitol, the initial concentrations of this solute were substantially lower than those of the other two, 0.8 mmol/L on the average, as compared with 2.5 and 2.4 mmol/L for erythritol and arabinose. However, there was sufficient loading to observe first order efflux kinetics over a 40-minute period.

### DISCUSSION

The results of these studies indicate that the increase in membrane permeability induced by deoxygenation of sickle cells has some selectivity. Acutely deoxygenated sickle cells showed the characteristic fivefold increase in ouabain-resistant Na+ and K+ fluxes under the conditions of these experiments, and a smaller but consistent twofold to threefold elevation in chloride influx when the anion transporter was inhibited with DIDS. However, they showed no detectable increase in permeability to sulfate anion or to three different neutral species, erythritol, mannitol, or arabinose. This pattern of permeability changes was different from the pattern of permeability changes induced by treatment of
normal cells with 2 mmol/L diamide. Although diamide treatment at this level gave the same increase in Na⁺ and K⁺ permeability observed in deoxygenated sickle cells, it also produced a twofold increase in the rate constants for efflux of mannitol and sulfate anion exchange in DIDS-inhibited cells, increases that were not produced by deoxygenation of sickle cells. Thus, it appears that the membrane defects in sickle cells and diamide-treated normal cells are different, despite the coupling of a permeability increase and an increase in transbilayer movement of phospholipids in both instances.

Joiner et al have also studied anion permeability in sickle cells, but they found no evidence for an effect of deoxygenation on either sulfate or chloride permeability.24,25 However, the experiments they performed to determine the effect of deoxygenation on chloride permeability at 37°C involved indirect measurements of K⁺ efflux from Valinomycin-treated cells. The assumption is that when K⁺ permeability is enhanced by Valinomycin treatment, the flux of K⁺ is limited by membrane permeability for the chloride counter-ion. A lack of effect of deoxygenation on the K⁺ efflux from Valinomycin-treated sickle cells was thus taken as an indication of a lack of effect on chloride permeability. At present, it is not clear how these results relate to our direct observations of an increase in chloride influxes associated with sickling.

Assuming that our observations reflect a real increase in chloride diffusional permeability in deoxygenated sickle cells, it suggests that the membrane leak is based primarily on solute size, with little discrimination on the basis of charge. This would be consistent with the observed increases in permeability to all the alkali metal cations, Ca²⁺, and chloride, which have similarly small hydrated radii, and the absence of increased permeability to erythritol, which is uncharged but substantially larger. The diamide-induced leak behaves rather differently, showing an increase in permeation of much larger solutes. It has been proposed that it can be represented by transient pores, of a size that admits passage of rather large solutes, albeit at a slower rate than small solutes. We speculate that the leak induced in sickle cells places a more stringent limit on the size of molecules that can pass through it.

Our results on the pattern of permeability changes induced by diamide differ somewhat from those reported by Deuticke et al.19 They found that the increase in permeability to erythritol, arabinose, and mannitol varied inversely with solute size, giving the largest magnitude of leak for erythritol. In contrast, we found increased permeability only for mannitol, the largest solute. Although we made an effort to use the same experimental conditions described by these investigators, it appears that in our experiments a much smaller change in fluxes occurred, such that the increase was only detectable for the solute with an extremely low background flux rate.

The present results confirm the observations of Joiner that DIDS treatment reduces the cation fluxes in deoxygenated sickle cells.26 Because DIDS, an amino reactive agent, reacts primarily with the anion transport protein band 3, it was originally proposed that the deoxygenation-induced cation leak might involve an altered form of the band 3 protein.17 However, subsequent work showing that the deoxygenation-induced leak and anion transport can be differentiated in their DIDS concentration and temperature dependence suggests that the two fluxes may use different pathways.24 Interestingly, the inhibitory effect of DIDS in deoxygenated sickled cells contrasts with its effect of enhancing cation fluxes in oxygenated sickle cells, and in both diamide-treated and untreated normal RBCs, suggesting a relatively specific effect.

In initiating these experiments, we hypothesized that the deoxygenation-induced leak was due to localized areas of mechanical stress on the membrane overlying spicules of polymerized Hb S. Our current observations, suggesting a charge-insensitive pathway with a stringent size limitation, are compatible with this possibility. However, Joiner has shown that DIDS suppresses most of the deoxygenation-dependent cation permeability change without reducing morphologic distortion. As proposed by Joiner, this may suggest an indirect effect of membrane stretching on permeability. Ney et al26 have reported that deformation of sickle cells by application of fluid shear stress also causes an increase in K⁺ permeability, which is synergistically enhanced under peroxidative stress by t-butylhydroperoxide. However, this type of mechanical stress is qualitatively different from the stress induced by polymer in the spicules. When cells are undergoing stable, shear-induced deformation, the stress is distributed over a large portion of the membrane surface as the cell membrane "tank-treads" around the interior. In contrast, in the sickled cell, stress appears to be focused on a relatively small and constant area. This difference could be the basis for the much larger K⁺ leak seen in deoxygenated sickle cells as compared with that in oxygenated sickle cells subjected to high shear stress. Finally, another recent finding should be considered in proposing a model involving permeability changes in the spicules. Liu et al have demonstrated that the membrane covering long spicules is essentially devoid of spectrin, although it retains the band 3 protein.27 Thus, in considering possible mechanisms for inducing a membrane permeability change, one should probably think of an RBC membrane stripped of its skeletal underpinnings rather than an intact RBC membrane. At present, little is known about the influence of the membrane skeleton on membrane permeability, and it is difficult to judge how DIDS treatment might interact with any such influence.

ACKNOWLEDGMENT

The authors are grateful to Dr William C. Mentzer and Klara Kleman for providing samples of sickle cells. This is paper no 101 from the MacMillan-Cargill Hematology Research Laboratory, University of California, San Francisco.

REFERENCES


8. Fabry ME, Nagel RL: The effect of deoxygenation on red cell density: Significance for the pathophysiology of sickle cell anemia. Blood 60:1370, 1982
23. Clark MR, Rossi ME: Deoxygenation-induced increase in sulfate efflux from DIDS treated sickle red cells. Blood 70:60a, 1987 (suppl 1)
27. Liu SC, Derick LH, Zhai S, Palek J: Ultrastructural anatomy of the red cell membrane lesion in sickle cells: Penetration of the hemoglobin S polymers through the membrane skeleton and reorganization of the skeletal lattice. Blood 74:44a, 1989 (suppl 1)
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