Oral Magnesium Supplements Reduce Erythrocyte Dehydration in Patients with Sickle Cell Disease

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Abstract

Intracellular polymerization and sickling depend markedly on the cellular concentration of sickle hemoglobin (Hb S). A possible therapeutic strategy for sickle cell disease is based on reducing the cellular concentration of Hb S through prevention of erythrocyte dehydration. The K-Cl cotransporter is a major determinant of sickle cell dehydration and is inhibited by increasing erythrocyte Mg content.

We studied 10 patients with sickle cell disease before treatment and after 2 and 4 wk of treatment with oral Mg supplements (0.6 meq/kg/d Mg pidolate). Hematological parameters, erythrocyte Na, K, and Mg content, erythrocyte density, membrane transport of Na and K, and osmotic gradient ektacytometry were measured. We found significant increases in sickle erythrocyte Mg and K content and reduction in the number of dense sickle erythrocytes. Erythrocyte K-Cl cotransport was reduced significantly. We also observed a significant reduction in the absolute reticulocyte count and in the number of immature reticulocytes. Ektacytometric analysis showed changes indicative of improved hydration of the erythrocytes. There were no laboratory or clinical signs of hypermagnesemia. Mild, transient diarrhea was the only reported side effect. We conclude that oral Mg supplementation reduces the number of dense erythrocytes and improves the erythrocyte membrane transport abnormalities of patients with sickle cell disease. (J. Clin. Invest. 1997. 100:1847–1852.) Key words: sickle cell • erythrocytes • anti-sickling agents • potassium • carrier proteins

Introduction

The delay time for sickle hemoglobin (Hb S) polymerization in Hb S–containing (SS) erythrocytes has an inverse exponential relationship to the cellular concentration of Hb S to its 20–40th power (1). The presence of intracellular Hb S polymers also depends on cellular Hb S concentration (2). Therefore, small changes in Hb S concentration affect markedly hemoglobin polymerization and cell sickling. One of the distinguishing characteristics of the sickle erythrocyte is the presence of dense erythrocytes that are formed as a consequence of cell dehydration and K loss. Dense erythrocytes are thought to play an important role in the vasoocclusive manifestations of this disease (3, 4).

Therapeutic strategies for sickle cell (homozygous for Hb S, or SS) disease aim at reducing Hb S polymerization. Hydroxyurea achieves this effect by increasing the cellular concentration of fetal hemoglobin (Hb F) which reduces Hb S polymerization to values seen in patients with the milder heterozygous for sickle and C hemoglobin (SC) disease (5–7). An additional therapeutic strategy for SS disease is based on reducing the cellular concentration of Hb S by preventing cell dehydration and K loss via the blockade of specific membrane cation transport systems. The two major pathways responsible for K loss and dehydration of SS erythrocytes are the Ca2+-activated K channel (Gardos) (8) and the K-Cl cotransport (9, 10). The imidazole antimycotic clotrimazole specifically induces blockade of the Gardos channel (11). With the use of this inhibitor, the role of the Gardos channel in SS erythrocyte dehydration has been demonstrated in vitro (12), in vivo in the SAD mouse, a transgenic model of SS disease (13), and in patients with SS disease (14).

We and others have explored the role of erythrocyte Mg in modulating the K-Cl cotransport system of normal (15) and SS erythrocytes in vitro (16, 17). Several reports have suggested that erythrocyte Mg content can be increased with dietary Mg supplementation in patients with diabetes (18, 19). We have shown recently that dietary Mg supplements improved erythrocyte abnormalities and increased hemoglobin in the SAD mouse (20). In this report, we test the hypothesis that dietary Mg supplementation may induce beneficial changes in SS erythrocyte dehydration in patients with SS disease.

1. Abbreviations used in this paper: ALT, serum glutamic pyruvate transaminase; AST, serum glutamic oxaloacetic transaminase; BUN, blood urea nitrogen; CBC, complete blood count; CH, hemoglobin content; CV, corpuscular volume; D50, median density; DI, deformability index; DW, distribution width; Hb F, fetal hemoglobin; Hb S, sickle cell hemoglobin; HC, hemoglobin concentration; HDW, hemoglobin DW; M, mean; O, osmolality value in the hyperosmolar region at which the DI reaches half the maximum value; r, reticulocyte; R50, median 60% density range; RDW, red cell volume DW; SC, heterozygous for Hb S and C hemoglobin; SS, homozygous for Hb S.
Methods

Patient selection. Individuals over 18 yr of age with SS disease were eligible. Inclusion criteria included normal renal and liver function, performance status of 70% or greater, and no blood transfusions during the preceding 3 mo. An investigator explained the study completely to each participant, who then gave written informed consent. A negative pregnancy test was required for female subjects. All the patients were treated as outpatients. No patients were hospitalized during the study.

Study protocol. In the initial phase, erythrocyte and plasma Mg levels were determined in 21 patients with SS disease, 24 patients with SC disease, and 17 normal controls.

11 of the SS patients were enrolled in the dietary Mg supplementation study. One of the patients did not return after the baseline studies and could not be reached for follow-up. 10 patients completed the Mg supplementation protocol.

The following studies were performed three times: at the time of entry, after 2 wk and after 4 wk of Mg therapy. For the dietary Mg intake, the daily Mg intake as mg per day in a 70-kg subject. The daily Mg intake of normal subjects estimated from dietary history was 418±120 mg for males and 343±94 mg for females (21).

Subjects were given 0.6 meq mg didaplate/kg body wt/d (MAG-2; Theraplix, Paris, France), divided into two oral daily doses, for 4 wk. This dosage corresponds to 504 mg of supplemental Mg per day in a 70-kg subject. The daily Mg intake of normal subjects estimated from dietary history was 418±120 mg for males and 343±94 mg for females (21).

The following variables were quantified: (a) O\textsubscript{max} the osmolality at which the deformability index (DI) reaches a minimum in the hypertonic region of the gradient, corresponds to the osmolality at which 50% of the red cells hemolyze in a standard osmotic fragility test. This index provides a measure of the average surface area-to-volume ratio of the erythrocytes. (b) DI\textsubscript{max} the maximum value of the DI attained at physiologically relevant osmolality, is quantitatively related to the mean surface area of red cells. (c) O\textsuperscript{'} the osmolality value in the hyperviscous region at which the DI reaches half the maximum value, provides information on the hydration state of the erythrocytes.

Erythrocyte composition, density, and ion transport studies. Erythrocyte cation content and erythrocyte density distribution curves, using phthalate esters, were determined as described previously (25). We evaluated three parameters in the phthalate esters assay (26): (a) D\textsubscript{H+}, (b) R\textsubscript{Mg}, or median density, the density value that divides the red cell population in half; (b) R\textsubscript{Mg}, or median density range obtained after subtracting the 20% lightest and densest fractions, quantifies the spread in cell densities which is characteristic of SS disease; and (c) percentage of dense cells, corresponding to the percentage of cells with density > 1.120. With these values and the absolute erythrocyte count obtained from the CBC, the number of so-defined dense cells per microliter can be calculated. Repeated measurements in different patients indicate that the reproducibility of these assays (expressed as mean±SD of the individual cell volume for eight patients) is 0.11±0.07% for D\textsubscript{H+} and 12.7±7.3% for the percentage of dense cells.

Plasma and buffy coat were removed after centrifugation at 1,200 g for 10 min, and the cells were washed four times with choline wash solution containing 152 mmol/liter choline chloride, 1 mmol/liter Tris-Mops, pH 7.4, at 4°C. Erythrocyte K and Na contents were determined with atomic absorption spectrometry. Repeated measurements in different patients indicate that the reproducibility of the erythrocyte K content assay (expressed as mean±SD of the individual cell volume for eight patients) is 4.6±2.7%.

K-Cl cotransport from fresh cells was measured as chloride-dependent net K efflux into hypotonic medium. Flux medium for chloride-dependent K efflux contained (in mmol/liter) 100 Na, 1 Mg (the anion either Cl\textsuperscript{−} or NO\textsubscript{3}−), 10 glucose and 10 Tris-Mops (pH 7.4 at 37°C). Chloride-dependent K efflux was calculated from the difference between K efflux into chloride and that into nitrate. Efflux rates were calculated from net flux measurements taken after 5 and 25 min of incubation at 37°C. Repeated measurements in different patients indicate that the reproducibility of these assays (expressed as mean±SD of the individual CV for eight patients) is 10.9±5.5%.

The maximal rates of Na-K pump and Na-K-2Cl cotransport systems were measured in cells containing equal amounts of Na and K (∼ 50 mmol/liter of cells each). Erythrocytes were treated with the nystatin technique to modify the intracellular cation composition (27). The nystatin-loading solution contained (in mmol/liter) 70 NaCl, 70 KCl, and 55 sucrose. Na-K pump was estimated as the ouabain-sensitive fraction on Na efflux into a medium containing (in mmol/liter) 130 choline chloride and 10 KCl. Duplicate samples were incubated for 5 min and 25 min at 37°C. The ouabain concentration was 0.1 mmol/liter. Na-K-2Cl cotransport was estimated as the bumetanide-sensitive fraction of Na efflux into a medium containing (in mmol/liter) 140 choline chloride and 0.1 ouabain. The efflux times were 5 and 25 min at 37°C with triplicate samples. The bumetanide concentration was 0.01 mmol/liter. All media contained (in mmol/liter) 1 MgCl\textsubscript{2}, 10 glucose, and 10 Tris-Mops (pH 7.4 at 37°C).

Osmotic gradient ektacytometry. We used osmotic gradient ektacytometry to quantitate cellular deformability, which is regulated by surface area, surface area-to-volume ratio, and state of hydration of red cells (28). We performed osmotic gradient ektacytometry on fresh blood samples in five of the patients enrolled in this trial, at baseline and after 4 wk of Mg supplementation. Two of these patients were also studied after 2 wk of therapy. We mixed erythrocytes continuously with a 4% polyvinylpyrroliodone solution of gradually increasing osmolality (from 60 to 450 mosM), and recorded the deformability index with an ektacytometer (Bayer Corp., Diagnostics Division) as a function of osmolality at a constant applied shear stress of 170 dynes/cm². The following variables were quantified: (a) O\textsubscript{max} the osmolality at which the deformability index (DI) reaches a minimum in the hypertonic region of the gradient, corresponds to the osmolality at which 50% of the red cells hemolyze in a standard osmotic fragility test. This index provides a measure of the average surface area-to-volume ratio of the erythrocytes. (b) DI\textsubscript{max} the maximum value of the DI attained at physiologically relevant osmolality, is quantitatively related to the mean surface area of red cells. (c) O\textsuperscript{'} the osmolality value in the hyperviscous region at which the DI reaches half the maximum value, provides information on the hydration state of the erythrocytes.

Osmotic gradient ektacytometry quantitates the surface area-to-volume ratio, the membrane surface area, and the state of hydration of erythrocytes in the blood sample. Normal ranges for these different parameters had been established in our laboratory with blood samples from 144 healthy adult blood donors (29).

Erythrocytes of patients with SS disease show, to a variable extent, a left shift in O\textsubscript{max}, a left shift in O\textsuperscript{'} indicative of red cell dehydration, and a decrease in DI\textsubscript{max}, which most likely reflects heterogeneity in cell water content rather than a uniform reduction in surface area.
**Results**

**Serum and erythrocyte Mg in SS and SC disease.** Fig. 1 presents data on the erythrocyte Mg content of patients with SS or SC disease and normal control subjects. The SS and SC erythrocytes have a reduced erythrocyte Mg content compared with normal controls (in mmol/kg hemoglobin, 6.6±1.2, n = 21, 6.2±0.9, n = 24, and 8.62±0.8, n = 17, P < 0.05, respectively). Measurements of plasma Mg showed no differences between SS and SC patients and normal controls (data not shown).

The effect of dietary Mg supplementation on SS erythrocyte Mg content. Table I presents data obtained from measurements of plasma and erythrocyte Mg in the 10 SS disease patients treated with Mg pidolate. Dietary Mg supplementation did not affect plasma Mg, which remained unchanged from baseline. A significant increase in erythrocyte Mg content was observed after 14 and 28 d of Mg supplementation, with values similar to those of normal cells at day 14, but somewhat higher than normal cells at day 28. These changes are much greater than those obtained with slightly lower doses of Mg pidolate in subjects with diabetes mellitus, who had normal baseline erythrocyte Mg content (18, 19).

Statistical analysis. All values are means±SD. Comparisons of separate variables between baseline state and values after 14 and 28 d of treatment were performed using two-tailed Student's t test. Comparison of more than two groups was performed by one-way ANOVA with Tukey's test for post hoc comparison of the means (30).

![Figure 1. Erythrocyte Mg content (mean±SD) in normal controls (AA, n = 17) and in untransfused patients with SS (n = 21) and SC (n = 24) disease.](image)

![Figure 2. Erythrocyte cation content in patients with SS disease at baseline and after 14 and 28 d of oral Mg pidolate supplements (0.6 meq/kg body wt/d, n = 10). *P < 0.05 and °P < 0.005.](image)

Table I. Plasma and Erythrocyte Mg at Baseline and during Dietary Mg Supplementation

<table>
<thead>
<tr>
<th>Time</th>
<th>Plasma Mg (mM)</th>
<th>Erythrocyte Mg (mmol/kg Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.862±0.057 (11)</td>
<td>5.18±0.24 (11)</td>
</tr>
<tr>
<td>14</td>
<td>0.954±0.11 (10)</td>
<td>9.34±2.3 (10)*</td>
</tr>
<tr>
<td>28</td>
<td>0.937±0.09 (10)</td>
<td>11.4±1.2 (1)°</td>
</tr>
</tbody>
</table>

Patients were treated with oral Mg pidolate at 0.6 meq/kg body wt/d. Data are presented as means±SD (n of determinations). *P < 0.05 and °P < 0.005 compared to baseline.
studies do not allow ruling out possible effects of Mg on cell deformability that are independent of the improved hydration state.

The effects of dietary Mg supplementation on hematological indices for erythrocytes and reticulocytes. Table II presents results obtained from the determination of several hematological and biochemical parameters. A trend for an increase in hemoglobin levels did not reach statistical significance. No significant changes occurred in MCV, MCHC, and MCH, nor in the distribution widths RDW and HDW and values for MCH (data not shown).

A significant reduction occurred in the absolute reticulocyte count after 28 d (Table II). This reduction was associated with a significant reduction in the absolute number of reticulocytes with high staining intensity, which corresponds to the most immature fraction of reticulocytes, and which has the highest residual RNA content. No significant changes occurred in the absolute number of reticulocytes with low and medium staining intensity (data not shown). Other cellular characteristics of the reticulocytes were unchanged, such as reticulocyte cell volume (MCV$: 113\pm7.6, 107\pm4.4, \text{and } 108.8\pm5.3$, at baseline, 14, and 28 d, respectively) and reticulocyte cell hemoglobin concentration (CHCM$: 26.3\pm2.1, 27.4\pm1.5$, and $27\pm1.0$ at baseline, 14, and 28 d, respectively).

Table II additionally shows significant changes in the density of the SS erythrocytes with Mg therapy. The median density of erythrocytes fell significantly after 14 and 28 d of dietary Mg supplementation (Table II). The middle density range ($R_60$), which quantifies the heterogeneous distribution of erythrocyte densities, was also reduced significantly with dietary Mg supplementation.

Fig. 5 shows that a significant reduction in the absolute number of dense, dehydrated SS erythrocytes, was observed after 14 and 28 d of Mg supplementation. These data were obtained with the phthalate density profile method, which quantifies cells with density $>1.120$. Estimates of dense cells based on the Bayer H*3 analyzer, which quantifies erythrocytes with a CHCM value $>38$ g/dl, also showed a significant reduction of dense cells after 28 d of Mg therapy (data not shown).

Clinical and side effects of dietary Mg supplementation. No significant side effects of dietary Mg supplementation were noted in this trial, with the exception of transient diarrhea in 1 of the 10 patients. No significant changes were observed in plasma levels of ALT, AST, or indirect bilirubin (not shown).

Figure 3. Activity of erythrocyte K-Cl cotransport (A) and Na-K pump (B) at baseline, and after 14 and 28 d of oral Mg pidolate supplements (0.6 meq/kg body wt/d, $n=10$) in patients with SS disease. *$P<0.05$ and **$P<0.005$.

Figure 4. Osmotic deformability profile for erythrocytes collected from a patient with SS disease at baseline and after 14 and 28 d of oral Mg pidolate supplementation (0.6 meq/kg body wt/d). A profile of normal control cells is also presented. A significant shift in O’ indicates an improved hydration state of the SS erythrocytes after Mg supplementation.
Plasma Mg, BUN, and creatinine did not change during Mg supplementation (Table I, and data not shown).

**Discussion**

The role of erythrocyte Mg content in regulating K-Cl cotransport had been demonstrated in erythrocytes of normal human controls (15) and of patients with SS disease (16, 17). Oral administration of Mg has been shown to reduce dehydration and increase hemoglobin levels in the SAD mouse, a transgenic murine model of SS disease (20). Studies in diabetic patients have shown that dietary Mg supplements can increase erythrocyte Mg content (18, 19). Based on this preliminary evidence, we tested the hypothesis that dietary Mg supplements could increase erythrocyte Mg, inhibit K-Cl cotransport, and reduce dehydration in patients with SS disease.

We found erythrocyte Mg content of SS and SC patients to be reduced compared with normal controls (Fig. 1). A large study also reported lower levels of erythrocyte Mg in SS disease (32). Most of the Mg contained in erythrocytes is bound to cellular proteins, ATP, and 2,3-diphosphoglycerate. The normal erythrocyte membrane is functionally impermeable to Mg. The Na/Mg exchanger, which extrudes Mg from the cell in exchange for Na, provides the only known mechanism for Mg loss in normal erythrocytes (33), but remains poorly understood. This system most likely mediates the decrease in erythrocyte Mg observed with cell age (or density): a two to fourfold difference in cell Mg has been described between high and low density fractions of human erythrocytes (34, 35). A preliminary report of increased activity of the Na/Mg exchanger in Mg-loaded SS erythrocytes (36) might provide an explanation for the reduced Mg content of SS cells. The reduction in total cell Mg content is evident particularly in dense SS erythrocytes. In addition, these cells exhibit an abnormal increase in free Mg due to reduction in the intracellular Mg buffering capacity (31). This may promote further Mg loss upon sickling, because the free Mg gradient is outwardly directed, and sickling has been shown to increase the permeability of the erythrocyte membrane to Mg (31). These data indicate that the reduced erythrocyte Mg of SS erythrocytes can be restored to normal or above normal values in patients treated with dietary Mg supplements (Table I). The intracellular distribution of Mg was not studied in these patients. Thus, we cannot estimate the relative magnitude of the changes in free erythrocyte Mg.

The normalization of erythrocyte Mg content is associated with a significant reduction in K-Cl cotransport activity and with increased K content. The reduced K-Cl cotransport can be interpreted as a direct effect of increased Mg content, although indirect effects are possible, including reduction in absolute reticulocyte number or diminished oxidative damage. K-Cl cotransport is the primary mechanism for dehydration of transferrin receptor–positive reticulocytes (37).

Mg is an important regulator of ion transport across cellular membranes. Mg is an essential cofactor for Na-stimulated phosphorylation of the Na-K ATPase (38). A [Mg]/[ATP] ratio near one is optimal for Na-K pump activity, with inhibition at higher and lower ratios. It has been demonstrated that the reduced Na-K pump activity of dense SS erythrocytes can be normalized when a proper [Mg]/[ATP] ratio is restored (31). We interpret the observed changes in the maximal rate of the Na-K pump (Fig. 3) as an indication that the optimal [Mg]/[ATP] ratio has been restored with dietary Mg supplements, although cell ATP content was not measured in this study.

A finding shared by this clinical trial and our recently published report on the use of clotrimazole in SS disease (14) is the observation of a reduction in the number of circulating dense SS erythrocytes. These two studies together indicate that both the Gardos channel and the K-Cl cotransporter are involved in the in vivo generation of dense SS cells, as recent in vitro studies suggested (39).

Several uncontrolled studies have reported beneficial ef-

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**Table II. Hematological Parameters in Sickle Cell Patients at Baseline and during Mg Supplementation**

<table>
<thead>
<tr>
<th>Time</th>
<th>Hb (g/dl)</th>
<th>MCV (fl)</th>
<th>MCHC (g/dl)</th>
<th>Retic.</th>
<th>HRetic.</th>
<th>Dretic.</th>
<th>Retic.%</th>
<th>Dretic.%</th>
<th>10^11/μl</th>
<th>Dg/kg/d</th>
<th>O' (mosM kg⁻¹)</th>
</tr>
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<tbody>
<tr>
<td>Baseline</td>
<td>8.1 ± 1.0</td>
<td>90.2 ± 9.0</td>
<td>33.2 ± 1.4</td>
<td>285 ± 72</td>
<td>30.2 ± 10.2</td>
<td>1.098 ± 0.002</td>
<td>0.013 ± 0.003</td>
<td>296.4 ± 20.1</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>14</td>
<td>8.2 ± 1.0</td>
<td>89.5 ± 8.9</td>
<td>33.5 ± 1.5</td>
<td>269 ± 73.5</td>
<td>30.6 ± 18.8</td>
<td>1.091 ± 0.005⁵</td>
<td>0.010 ± 0.004*</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>8.5 ± 1.1</td>
<td>90.9 ± 9.0</td>
<td>32.4 ± 0.8</td>
<td>228 ± 34.3*</td>
<td>23.9 ± 6.8*</td>
<td>1.092 ± 0.004⁴</td>
<td>0.010 ± 0.001*</td>
<td>306.4 ± 15.3*</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Patients were treated with oral Mg pidolate at 0.6 meq/kg/d. Data are presented as mean ± SD (n = 10). *P < 0.05 and †P < 0.005 compared to baseline. Retic., reticulocytes. Hretic., high staining intensity reticulocytes. ND, not determined.
fects of oral administration of Mg citrate or intravenous ad-
istration of Mg sulfate in patients with SS disease (40, 41). However, a 7-d course of oral Mg supplementation using Mg
 citrate did not change erythrocyte survival in three patients
with SS disease (42).
Although no changes in Hb levels were demonstrated in this
study, the reduction in both absolute reticulocyte counts and
percentage of immature reticulocytes induced by Mg ther-
apy suggests a possible improvement of the anemia. However,
an effect of the increased Mg content on reticulocyte matura-
tion cannot be ruled out. The improved erythrocyte deform-
ability observed in this study could also result in improved sur-
vival or oxygen delivery.
This study provides the first in vivo evidence for a role of
K-Cl cotransport in SS erythrocyte dehydration. The data jus-
tify proposing oral Mg pidolate as a potential therapeutic strat-
egy for preventing erythrocyte dehydration in SS disease.

Acknowledgments
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References
Sci. 565:75–82.
consequence of the heterogeneous distribution of hemoglobin types in sickle
5. Platt, O.S., S.H. Orkin, G. Dover, G.P. Beardsley, R. Miller, and D.G.
Nathan. 1984. Hydroxurea enhances fetal hemoglobin production in sickle cell
Eckert, R.P. McMahon, and D.R. Bonds. 1995. Effect of hydroxurea on the
A multiparameter analysis of sickle erythrocytes in patients undergoing hy-
8. Gordo, G. 1958. The function of calcium in the potassium permeability
rocyte cation and water content in sickle cell anemia. Science (Wash. DC). 232:
388–390.
11. Alvarez, J., M. Montero, and J. Garcea-Sanchez. 1992. High affinity inhibi-
tion of Ca2+-dependent K+ channels by cytochrome P-450 inhibitors. J. Biol.
Ca2+-dependent K+ transport and cell dehydration in sickle erythrocytes by clo-
13. De Franceschi, N., L. Sadane, M. Trudel, S.L. Alper, C. Brugnara, and
Y. Beuzard. 1994. Treatment with oral clotrimazole blocks Ca2+-activated K+
transport and reverses erythrocyte dehydration in transgenic SAD mice: a model
Alper, and O.S. Platt. 1996. Therapy with oral clotrimazole induces inhibition of
the Gardos channel and reduction of erythrocyte dehydration in patients with
cell density in human erythrocytes. Am. J. Physiol. (Cell Physiol.) 252:C269–
C276.