Zinc Deficiency in Sickle Cell Disease

Ananda S. Prasad,1 Eric B. Schoomaker, Jesus Ortega, George J. Brewer, Donald Oberleas, and Fred J. Oelshlegel, Jr.

Clinical similarities between patients with sickle cell anemia and zinc-deficient subjects suggested a secondary zinc deficiency in sickle cell anemia. Zinc was assayed in various biological fluids and tissues by atomic absorption spectrophotometry. Zinc in the plasma, erythrocytes, and hair was decreased and urinary zinc excretion was increased in anemia patients as compared to controls. Erythrocyte zinc and daily urinary zinc excretion were inversely correlated in the anemia patients \( (r = -0.63, P < 0.05) \), suggesting that hyperzincuria may have caused zinc deficiency in these patients. Carbonic anhydrase, a zinc metalloenzyme, correlated significantly with erythrocyte zinc \( (r = 0.94, P < 0.001) \). Plasma RNase activity was significantly greater in anemia subjects than in controls. We administered zinc sulfate, 660 mg per day, orally, to seven men and two women with sickle cell anemia. Two 17-year-old males gained 5 cm and 7 cm in height during 49 and 42 weeks of zinc therapy, respectively. All but one patient gained weight (0.5 kg to 4.1 kg). Five of the males showed increased growth of pubic, axillary, facial, and body hair, and in one a leg ulcer healed in six weeks on zinc and in two others some benefit of zinc therapy on healing of ulcers was noted.

Additional Keyphrases: treatment of side-effects of sickle cell anemia • trace elements • effects of chronic hemolysis • atomic absorption spectrophotometry • Zn in plasma, urine, hair and erythrocytes • carbonic anhydrase

Zinc is essential for man and its deficiency causes growth retardation, loss of appetite, and hypogonadism \( (1) \). Zinc is essential for the activity of a large number of enzymes \( (2) \) and recently its role in fibroplastic proliferation and collagen synthesis has been recognized \( (3) \). Studies by various investigators indicate that zinc is required for deoxyribonucleic acid (DNA) synthesis \( (4, 5) \) and DNA-dependent RNA polymerase (EC 2.7.7.6) has been demonstrated to be a zinc-dependent enzyme \( (6) \). Our most recent study has documented that thymidine kinase (EC 2.7.1.75) is a zinc-dependent enzyme and that its activity is adversely affected early by dietary lack of zinc \( (7) \), thus further establishing an enzymatic role of zinc in DNA synthesis.

Certain clinical features are common to some sickle cell anemia (SCA) patients and zinc-deficient patients, the latter as reported from the Middle East \( (1, 8) \). These symptoms include delayed onset of puberty and hypogonadism in the males, characterized by decreased facial, pubic, and axillary hair, short stature and low body weight, rough skin, and poor appetite. Inasmuch as zinc is an important constituent of erythrocytes, it appeared possible that long-continued hemolysis in patients with SCA might lead to a zinc-deficient state, which could account for some of the clinical manifestations mentioned above. Delayed healing of leg ulcers and the reported \( (9) \) beneficial effect of zinc therapy on leg ulcers in SCA patients would also appear to be consistent with the above hypothesis.

Here, we present data on zinc metabolism in patients with SCA and provide evidence of zinc deficiency in some patients with SCA. Preliminary results of the effect of zinc therapy in SCA patients are also presented.
Patients from the Detroit General Hospital Sickle Cell Anemia Adult Clinic were investigated. Eighteen men and 18 women comprised the group. The diagnosis of SCA was established by history, physical examination, and hematological studies, which included electrophoresis of hemoglobin on cellulose acetate and quantitative determination of A₂, F, and S hemoglobins. Hemoglobin electrophoresis revealed the S-S pattern; quantitation of A₂ hemoglobin gave normal values and F hemoglobin was less than 20% in all our cases. The controls were healthy, nonanemic Negro men and women ranging in age from 23 to 57 years. The ages of the SCA patients ranged from 15 to 69 years.

We took the usual precautions to avoid contamination with zinc during collection of samples throughout the study (10). Zinc was assayed with an atomic absorption spectrophotometer (Model 303; Perkin-Elmer Corp., Norwalk, Conn. 06856) according to methods published earlier (10). Plasma was diluted fourfold and assayed directly by using standards in glycerol:water (5:95 by vol). Undiluted samples of urine were assayed directly by atomic absorption spectrophotometry. Phosphates in the urine do not interfere with zinc assay by atomic absorption spectrophotometry (10). Washed erythrocytes were lysed with de-ionized water and then digested with concentrated HNO₃ before assay. Hair samples were washed with hexane and ethanol, dried overnight at 125 °C, and then digested with concentrated HNO₃ before assay.

RNase (EC 3.1.4.22) activity in plasma was assayed by a method reported previously (11). Red cell carbonic anhydrase (EC 4.2.1.1) protein and its isozymes I and II were measured by a radioimmunosorbent technique (12).

Plasma and erythrocyte zinc were also assayed in other anemic subjects, including patients with megaloblastic anemia, iron-deficiency anemia, and anemia associated with chronic diseases.

Seven men and two women with SCA were orally administered zinc sulfate, 660 mg daily, for various lengths of time. The clinical effects of this therapy are summarized below.

### Results

Of 18 men with SCA, 13 manifested signs of hypogonadism (decreased or absent facial, axillary, chest and pubic hair and delayed onset of puberty). In contrast, only four women showed decreased gonadal function, as suggested by late onset of menarche and menstrual abnormalities. This observation is consistent with the known greater susceptibility of male gonads to a lack of zinc.

The height and weight of the patients with SCA were compared with data obtained from the Ten State Nutrition Survey in 1968-70 (13). Two men with SCA were below the 10th height percentile, 14 were below the 15th weight percentile, and two were in the 50th weight percentile for their ages. Of 18 women, two were below the 10th height percentile, nine were below the 15th weight percentile, and four were in the 50th weight percentile for their ages.

Table 1 shows results of measurements of zinc in erythrocytes and hair in SCA patients and normal Negro controls. The erythrocyte zinc concentration was significantly smaller in SCA patients than in the controls. Of the 27 SCA patients whose erythrocytes were analyzed for zinc, six had very low values (more than 2 SD below the control mean) and four older subjects (over 30 years) had normal values. Erythrocyte zinc correlated with age (r = +0.58, P < .01), suggesting that older subjects had higher zinc content in their erythrocytes. The zinc concentration in the hair of SCA patients was decreased as compared to the controls. Mean plasma zinc concentrations in SCA patients were significantly less than those for healthy nonanemic controls. Plasma RNase activity was significantly higher in SCA patients than in the controls. The mean urinary excretion of zinc (per gram of creatinine) by SCA patients was greater than for the controls.

Figure 1 shows results of carbonic anhydrase determinations in relation to erythrocyte zinc concentrations in SCA patients. The values correlate closely (r = 0.94, P < .001). There was a significant negative correlation (r = −0.71, P < 0.05) between 24-h urinary zinc excretion and erythrocyte zinc values for SCA patients (Figure 2).

Table 2 shows results of zinc determinations in

### Table 1. Zinc in Plasma, Erythrocyte, Hair, and Urine, and RNase Activity in Plasma of Sickle Cell Anemia Patients and Controls

<table>
<thead>
<tr>
<th>Zinc in</th>
<th>Plasma, μg/dl</th>
<th>Erythrocytes, μg/g of Hb</th>
<th>Hair, μg/g</th>
<th>Urine, μg/g creatinine</th>
<th>Plasma RNase, μL/min per ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>112 ± 2.5 (23)</td>
<td>41.7 ± 1.2 (23)</td>
<td>193 ± 4.3 (17)</td>
<td>495 ± 35 (10)</td>
<td>0.310 ± 0.001 (16)</td>
</tr>
<tr>
<td>Patients</td>
<td>102 ± 2.7 (32)</td>
<td>35.2 ± 1.6 (27)</td>
<td>149 ± 10.3 (21)</td>
<td>739 ± 60 (12)</td>
<td>0.435 ± 0.002 (31)</td>
</tr>
<tr>
<td>P</td>
<td>&lt;.025 &lt;.01</td>
<td>&lt;.01 &lt;.01</td>
<td></td>
<td>&lt;.01 &lt;.01</td>
<td></td>
</tr>
</tbody>
</table>

± values are standard error. Numbers in parentheses are number of subjects. At least three 24-h urines were collected from each subject.
plasma and erythrocytes of persons with other anemias. In patients with untreated megaloblastic anemia, erythrocyte zinc was increased; in other anemic subjects the zinc values were unremarkable.

Figure 8 shows the change, during zinc therapy, in the height of two young men with SCA, both 17 years old at the time treatment was begun. The height of one increased 5 cm in 49 weeks and the other 7 cm in 42 weeks. The normal growth pattern of boys between 13 and 18 years is also shown in this figure. Normally, by age 17 the height curve shows a plateau.

Since the submission of this paper, both of these subjects have gained an additional 3 cm in height as of Nov. 1, 1974.

### Table 2. Plasma and Erythrocyte Zinc in Six Subjects with Anemias Other Than Sickle Cell Anemia

<table>
<thead>
<tr>
<th>Hb, g/l</th>
<th>Plasma, µg/dl</th>
<th>Erythrocytes, µg/g Hb</th>
<th>Diagnosis</th>
</tr>
</thead>
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<tr>
<td>68</td>
<td>128</td>
<td>55.7</td>
<td>Fe and folate deficiency</td>
</tr>
<tr>
<td>58</td>
<td>122</td>
<td>41.2</td>
<td>Fe deficiency</td>
</tr>
<tr>
<td>61</td>
<td>96</td>
<td>47.0</td>
<td>Fe deficiency</td>
</tr>
<tr>
<td>66</td>
<td>104</td>
<td>44.1</td>
<td>chr. urinary tract infection</td>
</tr>
<tr>
<td>90</td>
<td>112</td>
<td>50.6</td>
<td>megaloblastic anemia</td>
</tr>
<tr>
<td>90</td>
<td>106</td>
<td>57.0</td>
<td>folic acid deficiency</td>
</tr>
</tbody>
</table>

Controls
Mean 112 ± 11.8 41.7 ± 5.6 ± SD

Figure 4 shows the growth and development in the first of the patients referred to above. This patient grew pubic hair, and his external genitalia became more adult-like during 49 weeks of zinc therapy. His initial bone age was 11 years; after zinc therapy for 49 weeks, his bone age was 12.6 years. Before zinc therapy was begun, his skin appeared rough and a biopsy revealed mild parakeratosis. These skin abnormalities could not be detected after zinc administration.

Figure 5 shows the change in weight after zinc therapy, for all nine subjects. Except for one female who lost 0.5 kg during the observation period, all others gained weight.

Table 3 summarizes our results of zinc therapy with respect to growth of body hair in males. Five of seven males have shown a positive effect on growth of body hair; one male has been on zinc for only four weeks, rather too soon to show such an effect, and the other has not shown any effect of zinc on body hair growth so far.
Two males and one female in this group have had chronic leg ulcers for many years. In one male subject, the ulcers healed within six weeks of zinc therapy and in the cases of the other two, some benefit of zinc therapy on healing of ulcers has been noted.

Table 4 shows the changes in plasma and erythrocyte zinc values, and values for carbonic anhydrase and plasma RNase activity after zinc therapy. Plasma zinc increased in all subjects and a trend towards higher values for zinc in erythrocyte and carbonic anhydrase protein were observed in three subjects. Plasma RNase activity definitely decreased in five of seven subjects in which this was measured before and after zinc therapy.

**Discussion**

Our data show that some SCA patients are zinc deficient, although the severity varied considerably from one patient to another in this group. This conclusion is supported by our clinical and biochemical data. The older subjects showed higher erythrocyte zinc concentrations, the significance of which is not clear at present. Possible erythrocyte zinc concentrations may have some prognostic significance, but this must await future longitudinal clinical studies in a large group of subjects. A good correlation between erythrocyte zinc and carbonic anhydrase activity further supports our conclusion that tissue zinc was depleted in our subjects.

Plasma zinc alone is not a good indicator of a zinc-deficient state in patients such as these who continuously hemolyze; because the zinc content of erythrocytes is 14-fold that of plasma, values for plasma zinc would tend to be falsely elevated in such cases. In spite of this, the mean plasma zinc for our SCA patients was significantly lower than for the controls. Activity of RNase is inhibited by zinc and in zinc-deficient tissues its activity is increased (11). Thus an increased RNase activity in plasma of SCA patients may also indicate a zinc-deficient state.

Changes in the plasma and erythrocyte zinc concentrations similar to those observed in SCA patients were not seen in other anemic subjects in this study. Our data with respect to plasma and erythrocyte zinc in patients with megaloblastic anemia and iron deficiency anemia are similar to those previously reported by other investigators (14, 15).

Previously, one of us (16) reported results of plasma and erythrocyte zinc determinations in thalassemia patients from Egypt. Although both were decreased, the widespread nutritional deficiency of zinc in the Middle East makes it impossible to relate these changes to the chronic hemolytic state per se. Zinc metabolism should be studied in patients with other chronic hemolytic disorders, to establish whether or not continued hemolysis may lead to a zinc-deficient state in such patients.

In spite of tissue zinc depletion in SCA patients, the mean urinary excretion of zinc was higher than in
the controls. This may have been directly a result of increased filtration of zinc by the glomeruli, owing to continued hemolysis, or there may have been a defect in tubular re-absorption of zinc somehow related to SCA—a possibility that cannot be excluded at present. Continued hyperzincuria may have been responsible for tissue depletion of zinc as suggested by a significant negative correlation between values for 24-h urinary zinc excretion and erythrocyte zinc. At this stage, however, one cannot rule out additional factors such as predominant dietary use of cereal protein and other nutritional factors that affect zinc availability adversely, thus accounting for zinc deficiency (1, 8). Further work is warranted for proper elucidation of pathogenesis of zinc deficiency in SCA.

It is interesting to note that in SCA patients, the zinc concentration in the erythrocytes correlated well with the carbonic anhydrase content. We measured carbonic anhydrase protein rather than its activity in the erythrocytes; our data suggest that the synthesis of specific carbonic anhydrase apoenzyme depends on the availability of zinc and that this apoenzyme does not accumulate if zinc is lacking. This is consistent with our previous studies with respect to other zinc-dependent enzymes, in which we were unable to demonstrate in vitro stimulation of enzyme activities with zinc in zinc-deficient tissues (17). The mechanism by which zinc influences specific apoenzyme synthesis is unclear.

Our recent studies have demonstrated a potential beneficial effect of zinc on the sickling process, in vitro, mediated by its effect on the oxygen dissociation curve (18, 19) and the erythrocyte membrane (20). Thus it is suggested that SCA patients may derive potential benefits from zinc therapy in more ways than one. The results of our limited clinical trial with zinc therapy revealed beneficial effects of zinc on growth, development of secondary sexual characteristics in male subjects, and healing of chronic leg ulcers. These observations support our biochemical data that deficiency of zinc was a complicating factor in our SCA patients. Zinc therapy in such patients therefore should be considered. In addition to the above, we also noted apparent symptomatic improvement, presumably related to the anti-sickling effect of zinc, in our patients (20). Double-blind studies,

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however, must be done before any conclusion can be reached regarding symptomatic improvement.

We have observed no serious toxic side effects caused by zinc administration so far. Occasionally some patients have complained of nausea after taking zinc while they are in a fasting state. In general, zinc administration has been tolerated well by our patients. However, inasmuch as long-term effects of zinc administration in doses used in this study are not known, one must be cautious in treating SCA patients with zinc on a prolonged basis.

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References