Vitamin E deficiency in \( \beta \)-thalassemia major: changes in hematological and biochemical parameters after a therapeutic trial with \( \alpha \)-tocopherol\(^1\)\(^-\)\(^3\)

E. A. Rachmilewitz,\(^4\) A. Shifter, and I. Kahane

ABSTRACT Serum \( \alpha \)-tocopherol (vitamin E) levels less than 0.5 mg/100 ml were found in \( \beta \)-thalassemia major and intermedia. Vitamin E deficiency in thalassemia is not associated with malabsorption, but could be due to its consumption while neutralizing oxidative damage in red blood cell (RBC) membrane and other tissues. A therapeutic trial with vitamin E was carried out in eight patients with \( \beta \)-thalassemia major with 750 to 1000 IU/day for an average period of 16 months. Serum vitamin E levels were determined consecutively in these patients and in seven untreated patients throughout that period. The treated patients showed: 1) A 4-fold increase in both serum and RBC vitamin E levels; 2) serum vitamin E dropped to the low pretreatment values after discontinuation of the therapeutic trial; 3) decrease in abnormally high malondialdehyde levels, which were generated after oxidant stress and indicate peroxidative damage to the lipids portion of the RBC membrane; 4) no changes in the low titratable thiol groups calculated per RBC membrane protein; 5) in three of seven patients prolonged RBC survival was found after administration of vitamin E for 12 months; 6) no significant changes were found in transfusion requirements between the treated and untreated groups followed for a period of 16 and 18 months, respectively. While there seem to be practical and theoretical indications to treat thalasemic patients with vitamin E, this by itself is incapable of correcting the variety of changes in RBC membrane components. Am. J. Clin. Nutr. 32: 1850-1858, 1979.

The thalassemia syndrome is a congenital hemolytic anemia due to a genetic defect in the DNA or in messenger RNA directing the synthesis of one of the globin chains of hemoglobin (1). Very low and sometimes undetectable levels of serum \( \alpha \)-tocopherol (vitamin E) have been found in \( \beta \)-thalassemia major, also known as Cooley's anemia (2-5).

Low serum vitamin E levels have been found in several conditions associated with chronic steatorrhea, such as cystic fibrosis (6) and in prematurely delivered newborns (7), but this does not seem to be the case in thalassemia where there is no evidence in favor of malabsorption and particularly in lipid malabsorption as shown by the normal levels of \( \beta \)-carotene in the presence of severe vitamin E deficiency (4) and by normal absorption of retinol, triglycerides, D-xylene, and glucose (5). Alternatively, and in view of the data indicating lipid membrane peroxidation in red blood cells of patients with \( \beta \)-thalassemia major, it has been postulated that the low serum vitamin E levels may be due to a secondary consumption of the antioxidant consequent to the membrane oxidation rather than a primary cause in its metabolism and absorption (4). The ultimate conclusion of these observations justified a therapeutic trial with vitamin E to patients with \( \beta \)-thalassemia major, aiming at neutralizing the deleterious oxidative damage of the RBC membrane and eventual beneficial effect on red blood cell (RBC) survival, the severity of the anemia and the transfusion requirements.

\(^1\)From the Hematology Service, Hadassah University Hospital, Mt. Scopus, Jerusalem, and the Hebrew University Hadassah Medical School, Jerusalem, Israel.
\(^2\)Supported in part by a grant from F. Hoffmann-La Roche & Co., Ltd., Basle, Switzerland to E.A.R.
\(^3\)Address reprint requests to: E. A. Rachmilewitz, M.D., Hematology Service, Hadassah Hospital, Mount Scopus, Jerusalem, Israel.
\(^4\)Established investigator of the Chief Scientist's Bureau of the Israeli Ministry of Health.
The present report describes the results of such a therapeutic trial with its various effects on hematological and biochemical parameters in patients with severe β-thalassemia major.

Materials and methods

The following studies were performed on 15 Jewish patients from Kurdish extraction with βα- or ββ-thalassemia major. Most of them are regularly transfused every 4 to 8 weeks. The main clinical and hematological features of most of these patients have been reported in previous publications (8, 9). Blood samples were also obtained from a group of Arab and Jewish patients with β-thalassemia intermedia, who do not require frequent blood transfusions.

Fresh anticoagulated (ACD or heparin) or noncoagulated blood was obtained from all patients repeatedly, as late as possible after preceding transfusions every 4 to 8 weeks. Routine hematological examinations were carried out in each case according to standard methods using a coulter S (Coultar Electronics Ltd., Harpenden Herts. England). Serum or plasma were immediately separated and kept in 4°C no longer than 48 hr before analysis. Serum or plasma α-tocopherol (vitamin E) levels were determined according to the method of Hashim and Schutringer (10). Red cell vitamin E levels were estimated on freshly prepared RBC following the method of Kayden and Bjornson (11).

Red cell membranes were prepared as described by Kahane and Rachmilewitz (12). Susceptibility of membrane lipids to autoxidation after oxidative stress was based on the generation of malonyldialdehyde (MDA), a secondary breakdown product of lipid peroxidation, according to the method of Stocks and Dormandy (13). Determination of total titratable sulfhydryl groups were carried out in RBC membranes solubilized in 1% sodium dodecyl sulfate using the method of Ellman (14) with 5,5'-dithiobis(2-nitrobenzoic acid).

RBC survival was performed by measuring the halflife of autologous 51Cr labeled RBC before and after a therapeutic trial with vitamin E for a period of at least 12 months, following the recommendations of the International Committee for Standardization in Hematology (15). In each case the blood samples were collected daily for a minimum of 7 days. The survival was started after the same time interval after the most recent blood transfusion, before and after the therapeutic trial with vitamin E.

α-tocopheryl acetate (vitamin E), oil soluble, was prepared specifically for the present studies by Hoffman La Roche Inc., Nutley, N.J., and Basel, Switzerland in capsules containing 150 IU. The patients received a total dose of 1050 IU in divided doses three times daily for an average period of 16 months.

Results

Throughout the period of the trial in all the patients under study, there were no subjective or objective side effects that could have been attributed to vitamin E. Most of them claimed they felt better and demanded more capsules after the trial was discontinued.

Serum vitamin E levels

The mean serum or plasma (there were no differences in vitamin E levels determined either in serum or in plasma obtained in heparinized or ACD blood) vitamin E levels in eight untreated patients with βα or ββ thalassemia major calculated for 11 to 12 determinations in an average period of 16.25 months was 0.40 ± 0.1 mg/100 ml, which is below the low normal levels of 0.5 mg/100 ml (10, 16) while in treated patients the mean was 1.6 ± 0.2 mg/100 ml during an average period of 14.4 months (Table 1). In a few patients the levels were consistent throughout the period of study, while in some of them there were fluctuations in the monthly vitamin E determination as indicated by the relatively high standard deviation. The cause for these variations is not clear. The patients insisted that they took the vitamin in the recommended dose throughout the period of observation. The individual determinations in five treated and four untreated patients are shown in Figure 1. It can be seen that in the untreated patients almost all vitamin E levels throughout the period of observation were less than 0.5 mg/100 ml. In the treated patients most of the vitamin E levels were between 1.0 and 2.0 mg/100 ml. Four weeks after discontinuing the therapeutic trial, the first determination of serum vitamin E level showed low levels, similar to those found before the trial and in untreated patients (Fig. 1). The change in serum vitamin E levels possibly occurred much sooner since according to Kayden and Bjornson (11) and our observations, both RBC and serum vitamin E levels dropped almost to pretreatment levels within 48 to 72 hr after discontinuing oral administration of large quantities of vitamin E. Since most of the patients with β-thalassemia major are regularly transfused, which is a factor that may have an effect on serum vitamin E levels, a series of plasma vitamin E determinations were carried out in 16 patients with β-thalassemia intermedia, who do not require regular blood transfusions. As can be seen from Table 2 the mean serum vitamin E levels in these patients was 0.21 ± 0.1 mg/100 ml, which is even lower
<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Spleen</th>
<th>Type ( \beta )-thalassemia</th>
<th>Serum vitamin E levels (mg/100 ml)</th>
<th>Hemoglobin (g/100 ml)</th>
<th>No. transfusions/month</th>
<th>(^{14}C) RBC survival (days)</th>
<th>MDA* (nmol/g hemoglobin)</th>
<th>SH* (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>EAm</td>
<td>M</td>
<td>12</td>
<td>– ( \beta )</td>
<td>19</td>
<td>1.67 ± 0.35</td>
<td>7.0 ± 0.85</td>
<td>7.3 ± 0.9</td>
<td>0.73</td>
<td>1.05</td>
<td>9.0</td>
</tr>
<tr>
<td>ID</td>
<td>M</td>
<td>8</td>
<td>– ( \beta )</td>
<td>17</td>
<td>1.89 ± 0.7</td>
<td>7.3 ± 0.8</td>
<td>8.0 ± 0.7</td>
<td>0.58</td>
<td>0.76</td>
<td>13.5</td>
</tr>
<tr>
<td>LS</td>
<td>F</td>
<td>11</td>
<td>– ( \beta )</td>
<td>17</td>
<td>1.52 ± 0.36</td>
<td>6.4 ± 0.8</td>
<td>8.0 ± 0.69</td>
<td>0.65</td>
<td>0.9</td>
<td>19.0</td>
</tr>
<tr>
<td>MB</td>
<td>M</td>
<td>14</td>
<td>– ( \beta )</td>
<td>9</td>
<td>1.55 ± 0.9</td>
<td>6.6 ± 1.1</td>
<td>7.5 ± 0.75</td>
<td>0.85</td>
<td>1.07</td>
<td>13.0</td>
</tr>
<tr>
<td>MO</td>
<td>F</td>
<td>18</td>
<td>– ( \beta )</td>
<td>18</td>
<td>1.62 ± 0.1</td>
<td>6.1 ± 0.85</td>
<td>6.4 ± 0.87</td>
<td>0.94</td>
<td>1.2</td>
<td>14.0</td>
</tr>
<tr>
<td>NS</td>
<td>F</td>
<td>9</td>
<td>+ ( \beta )</td>
<td>6</td>
<td>1.45 ± 0.12</td>
<td>5.1 ± 0.99</td>
<td>6.9 ± 0.9</td>
<td>0.64</td>
<td>1.04</td>
<td>10.0</td>
</tr>
<tr>
<td>GZ</td>
<td>F</td>
<td>22</td>
<td>– ( \beta )</td>
<td>18</td>
<td>1.26 ± 0.26</td>
<td>6.8 ± 1.2</td>
<td>7.9 ± 1.0</td>
<td>0.46</td>
<td>0.65</td>
<td>7.2</td>
</tr>
<tr>
<td>GM</td>
<td>F</td>
<td>25</td>
<td>– ( \beta )</td>
<td>18</td>
<td>1.55 ± 0.60</td>
<td>7.6 ± 0.96</td>
<td>8.0 ± 0.64</td>
<td>0.46</td>
<td>0.65</td>
<td>11.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16 ± 3</td>
<td>1.6 ± 0.2</td>
<td>6.6 ± 0.77</td>
<td>7.5 ± 0.6</td>
<td>0.7 ± 0.2</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>EAr</td>
<td>M</td>
<td>13</td>
<td>– ( \beta )</td>
<td>21</td>
<td>0.56 ± 0.17</td>
<td>6.6 ± 0.93</td>
<td>8.3 ± 0.6</td>
<td>0.81</td>
<td>0.85</td>
<td>11.0</td>
</tr>
<tr>
<td>GD</td>
<td>M</td>
<td>18</td>
<td>– ( \beta )</td>
<td>24</td>
<td>0.53 ± 0.2</td>
<td>7.2 ± 0.9</td>
<td>7.5 ± 0.9</td>
<td>0.75</td>
<td>0.79</td>
<td>7.0</td>
</tr>
<tr>
<td>ZY</td>
<td>M</td>
<td>22</td>
<td>– ( \beta )</td>
<td>19</td>
<td>0.37 ± 0.07</td>
<td>7.5 ± 1.17</td>
<td>7.6 ± 1.5</td>
<td>0.42</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td>IR</td>
<td>F</td>
<td>25</td>
<td>– ( \beta )</td>
<td>12</td>
<td>0.34 ± 0.07</td>
<td>7.93 ± 1.23</td>
<td></td>
<td>0.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MA</td>
<td>M</td>
<td>16</td>
<td>– ( \beta )</td>
<td>14</td>
<td>0.35 ± 0.12</td>
<td>6.4 ± 1.3</td>
<td>6.9 ± 1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>12.0</td>
</tr>
<tr>
<td>SR</td>
<td>M</td>
<td>24</td>
<td>– ( \beta )</td>
<td>18</td>
<td>0.34 ± 0.12</td>
<td>7.1 ± 1.2</td>
<td>7.3 ± 1.0</td>
<td>1.2</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>M</td>
<td>20</td>
<td>+ ( \beta )</td>
<td>17</td>
<td>0.36 ± 0.10</td>
<td>7.8 ± 0.86</td>
<td>8.3 ± 0.56</td>
<td>0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>18 ± 4</td>
<td>0.4 ± 0.1</td>
<td>7.1 ± 0.53</td>
<td>7.7 ± 0.5</td>
<td>0.8 ± 0.3</td>
<td>0.8 ± 0.34</td>
</tr>
<tr>
<td>Normal Values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.5–1.0 (10, 16)</td>
<td>28.0 ± 0.5 (15)</td>
<td>120–150 (13)50–90 (12)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Average of 11 to 12 determinations during an average period of 16 months.  
* Determination between 6 to 12 months after vitamin E administration.
than in the transfused patients (Table 1). Serum vitamin E levels were measured in five treated and five untreated patients before, and immediately after blood transfusion and these levels were similar. The majority of patients with \(\beta\)-thalassemia major were splenectomized, and, therefore, plasma vitamin E levels were also determined in five normal adults who underwent traumatic splenectomy and the mean was 0.68 ± 0.076 mg/100 ml which is within the normal range (10, 16).

**RBC Vitamin E levels**

RBC vitamin E levels were examined in four normal controls, three untreated, and six treated patients. The levels in the normal controls were similar to those reported previously (11). In the untreated patients the levels were similar to the normal values. In all treated patients RBC vitamin E levels were 3- to 6-fold higher than the levels in the controls (Table 3). In four normal controls vitamin E levels were measured in isolated RBC membranes. The results were the same obtained for whole RBC, with an average of 3.6 ± 0.4 and 3.2 ± 5 \(\mu\)g/ml, respectively.

**Biochemical parameters**

As can be seen from Table 1, the average MDA levels generated in vitro following exogenous oxidant stress in the treated patients, was much less than the levels in the untreated patients. The differences between the two groups were statistically significant (\(P < 0.05\)). Moreover, in six out of eight treated patients single MDA values were measured before the onset of the clinical trial and the average value was 458 ± 131 nmole/g hemoglobin, when compared to the average value of all determinations in the treated

---

**FIG. 1.** Serial determinations of serum vitamin E levels in patients with \(\beta\)-thalassemia major treated and untreated with vitamin E. Arrows indicate the time when vitamin E administration was discontinued. Normal range of serum vitamin E levels is shown in the area with diagonal lines. (Date of five treated and four untreated representative patients.)
patients throughout the period of the trial, which was 237 ± 100 nmole/g hemoglobin. The results of these studies indicate decreased RBC membrane peroxidation in treated patients. To substantiate this observation a comparison was made between MDA levels in two siblings with $\beta^+$ thalassemia, (one (EA)) was treated with vitamin E, and the other (EA) received similar looking placebo tablets. The results, as shown in Figure 2, clearly indicate that in the treated sibling MDA levels were almost within the normal range, while in the untreated sibling MDA levels were 3- to 4-fold higher than the normal values.

However, there were no changes in the decreased titratable SH groups in RBC membrane preparations in patients who received vitamin E for at least 12 months with an average of 24.9 ± 9.1 nmole/mg protein when compared to previous results on the same patients and others before the present trial was underway, where the average was 26 ± 11.1 nmole/mg protein (12). These results indicate that excess of vitamin E seems to have no protective effect on the degree of membrane protein oxidation, as demonstrated by determination of at least one parameter.

**RBC survival**

RBC survival before and after vitamin E treatment was performed in seven treated and two untreated patients, since two subjects had initial studies only. Data were also available from an additional pair of siblings who received vitamin E periodically and, therefore, were not included in Table 1. The results indicate that while in three treated patients the survival was prolonged and in another patient only slightly prolonged after 12 months of vitamin E administration, there

---

**TABLE 2**
Determinations of serum vitamin E levels in patients with $\beta$-thalassemia intermedia (untransfused)

<table>
<thead>
<tr>
<th>Name</th>
<th>Age</th>
<th>Sex</th>
<th>Spleen</th>
<th>Hemoglobin</th>
<th>Fetal hemoglobin</th>
<th>Hemoglobin A$_2$</th>
<th>Serum vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>g/100 ml</td>
<td>%</td>
<td>%</td>
<td>mg/100 ml</td>
</tr>
<tr>
<td>AT</td>
<td>25</td>
<td>M</td>
<td>-</td>
<td>9.5</td>
<td>12.6</td>
<td>5.6</td>
<td>0.02</td>
</tr>
<tr>
<td>SA*</td>
<td>18</td>
<td>M</td>
<td>-</td>
<td>8.6</td>
<td>27.0</td>
<td>4.6</td>
<td>0.25</td>
</tr>
<tr>
<td>SA*</td>
<td>15</td>
<td>F</td>
<td>-</td>
<td>9.5</td>
<td>36.1</td>
<td>4.8</td>
<td>0.25</td>
</tr>
<tr>
<td>HA</td>
<td>14</td>
<td>F</td>
<td>-</td>
<td>9.4</td>
<td>11.0</td>
<td>4.2</td>
<td>0.23</td>
</tr>
<tr>
<td>SI*</td>
<td>16</td>
<td>M</td>
<td>-</td>
<td>8.4</td>
<td>1.9</td>
<td>7.6</td>
<td>0.1</td>
</tr>
<tr>
<td>SP*</td>
<td>18</td>
<td>M</td>
<td>-</td>
<td>8.1</td>
<td>25.2</td>
<td>6.3</td>
<td>0.39</td>
</tr>
<tr>
<td>SD*</td>
<td>7</td>
<td>F</td>
<td>-</td>
<td>7.6</td>
<td>23.5</td>
<td>6.1</td>
<td>0.28</td>
</tr>
<tr>
<td>ZH</td>
<td>24</td>
<td>M</td>
<td>-</td>
<td>9.1</td>
<td>6.4</td>
<td>9.7</td>
<td>0.23</td>
</tr>
<tr>
<td>HS</td>
<td>22</td>
<td>M</td>
<td>-</td>
<td>8.1</td>
<td>90.0</td>
<td>4.0</td>
<td>0.25</td>
</tr>
<tr>
<td>KA'</td>
<td>22</td>
<td>M</td>
<td>-</td>
<td>8.0</td>
<td>12.0</td>
<td>7.6</td>
<td>0.13</td>
</tr>
<tr>
<td>KA'</td>
<td>12</td>
<td>M</td>
<td>+</td>
<td>7.8</td>
<td>8.2</td>
<td>6.8</td>
<td>0.17</td>
</tr>
<tr>
<td>KM*</td>
<td>14</td>
<td>F</td>
<td>+</td>
<td>7.5</td>
<td>4.8</td>
<td>7.9</td>
<td>0.17</td>
</tr>
<tr>
<td>MSh</td>
<td>30</td>
<td>M</td>
<td>+</td>
<td>10.7</td>
<td>24.5</td>
<td>4.2</td>
<td>0.05</td>
</tr>
<tr>
<td>AN</td>
<td>35</td>
<td>M</td>
<td>+</td>
<td>10.5</td>
<td>19.8</td>
<td>7.0</td>
<td>0.16</td>
</tr>
<tr>
<td>NF*</td>
<td>13</td>
<td>F</td>
<td>+</td>
<td>8.3</td>
<td>20.8</td>
<td>6.8</td>
<td>0.3</td>
</tr>
<tr>
<td>DH</td>
<td>5</td>
<td>M</td>
<td>+</td>
<td>8.6</td>
<td>17.2</td>
<td>5.1</td>
<td>0.32</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td>8.73 ± 0.96</td>
<td>21.3 ± 20.6</td>
<td>6.0 ± 1.7</td>
<td>0.21 ± 0.1</td>
</tr>
</tbody>
</table>

* Patients with same superscript letter are siblings.

**TABLE 3**
Red cell vitamin E levels in patients with $\beta$-thalassemia major treated and untreated with vitamin E

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Serum</th>
<th>RBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mg/100 ml</td>
<td>$\mu$g/ml</td>
</tr>
<tr>
<td>Treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS</td>
<td>F</td>
<td>8</td>
<td>1.90</td>
<td>13.9</td>
</tr>
<tr>
<td>GZ</td>
<td>F</td>
<td>19</td>
<td>1.90</td>
<td>19.4</td>
</tr>
<tr>
<td>GM</td>
<td>F</td>
<td>22</td>
<td>2.00</td>
<td>11.6</td>
</tr>
<tr>
<td>YD</td>
<td>M</td>
<td>6</td>
<td>1.80</td>
<td>20.9</td>
</tr>
<tr>
<td>EA</td>
<td>M</td>
<td>11</td>
<td>1.70</td>
<td>11.3</td>
</tr>
<tr>
<td>YZ</td>
<td>F</td>
<td>23</td>
<td>1.85</td>
<td>12.9</td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MA</td>
<td>M</td>
<td>12</td>
<td>0.4</td>
<td>0.8</td>
</tr>
<tr>
<td>ZY</td>
<td>M</td>
<td>19</td>
<td>0.35</td>
<td>3.6</td>
</tr>
<tr>
<td>SI</td>
<td>F</td>
<td>12</td>
<td>0.35</td>
<td>2.6</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NB</td>
<td>F</td>
<td>24</td>
<td>0.65</td>
<td>3.0</td>
</tr>
<tr>
<td>IK</td>
<td>M</td>
<td>37</td>
<td>1.00</td>
<td>3.3</td>
</tr>
<tr>
<td>EAR</td>
<td>M</td>
<td>40</td>
<td>0.85</td>
<td>3.3</td>
</tr>
<tr>
<td>ZM</td>
<td>M</td>
<td>33</td>
<td>0.80</td>
<td>1.8</td>
</tr>
</tbody>
</table>
were no changes in the RBC survival of three other treated patients during the same period. Similarly, no changes in RBC survival were found in the other pair of siblings where the values were 10.5 and 10.5 compared to 14.0 and 10.2 days respectively, after a period of 12 months. Thus, although in a few patients administration of vitamin E resulted in prolonged half life of $^{51}$Cr tagged RBC, the results were not uniform for the entire treated group. It is noteworthy that the RBC survival in some of the treated as well as the untreated patients was almost the same after a time interval of 12 months.

**Changes in transfusion requirements**

At the onset of the therapeutic trial with vitamin E it was decided to keep pretransfusion hemoglobin levels around 7.5 g/100 ml in both treated and untreated patients. For that reason, in a few treated patients there was an average increase in both pretransfusion hemoglobin level and in transfusion requirements when compared to the pretreatment period. As shown in Table 1 there was no significant difference in the transfusion requirements between the treated and untreated group of patients. There were also no changes in the transfusion requirements in the untreated group followed for a similar period of time before and after the onset of the therapeutic trial.

**Discussion**

There is no specific therapy for the severe forms of thalassemia and the major symptomatic treatment consists of regular blood transfusions once every few weeks with the consequent development of severe iron overload terminating in fibrosis, necrosis and impaired functioning of major organs (1). One of the reasons for the marked tissue abnormalities in diseases complicated by iron overload, such as thalassemia, is believed to be due to free oxygen radical formation by a chain reaction that is started by the ions of transition metals such as iron (17). Moreover, it has been shown that more superoxide radical is generated following in vitro oxidation of separated hemoglobin subunits (18), a situation that resembles the intracellular environment in a thalassemic RBC. These radicals, also designated "activated oxygen" (19) are thought to play a major role in RBC destruction and hemolysis, by reacting with labile polyunsaturated fatty acids, having reactive methylene groups, in the RBC membrane (17) and possibly in membranes of other major organs. The finding of decreased levels of polyunsaturated fatty acids (4) and the increased generated MDA levels after exogenous oxidative stress with H$_2$O$_2$ in thalassemic RBC (3) confirmed this assumption.

Vitamin E is known to protect a nonenzy-
matic attack of molecular oxygen on poly-
unsaturated fatty acids (20) and therefore
vitamin E status in the severe forms of the
thalassemia syndrome has been investigated.
In three reports from different parts of the
world low serum vitamin E levels (<0.5 mg/
100 ml) were found in β-thalassemia major:
seven out of 21 patients (33%) in a group who
were regularly transfused (3), 46% of 56 pa-
tients in a previous report and 50% of 18
patients in a recent report, who were inade-
quately transfused (2, 5) and also in 26 of 27
patients who are moderately transfused (4).
In order to find the genuine vitamin E status
in thalassemia major without any possible
influence caused by donor blood, serum vi-
tamin E levels were determined in 16 patients
with β-thalassemia intermedia, who have a
significant degree of anemia but do not re-
quire frequent blood transfusions. The results
showed that serum vitamin E levels were
found to be markedly decreased in all of them
(Table 2). Low serum vitamin E levels could
not be related to the variable percentage of
fetal hemoglobin and hemoglobin A₂ and
whether the patients were splenectomized.
In addition, the results were not influenced by
the dietary habits of the two different ethnic
groups of Jews and Arabs. The reason for the
lower serum vitamin E levels in untransfused
patients is not clear. It is possible that normal
donor RBC, which are added regularly to the
more severe patients, have less demand for
the protective antioxidant effects of vitamin
E.

Unlike other conditions where vitamin E
deficiency has been documented, which were
primarily related to intestinal malabsorption,
there is no clinical evidence for malabsorp-
tion in thalassemia, where β-carotene, retinol,
triglycerides, D-xylose, and glucose absorp-
tion were found to be normal (4, 5). Changes
in serum lipid levels may affect α-tocopherol
levels, and low tocopherol levels may not
always indicate tissue depletion of tocopherol
(21). In β-thalassemia major serum total lipid
levels were significantly lower than in normal
controls (5), probably due to the presence of
both chronic anemias (22) and liver damage
(23). However, the mean ratio of serum vi-
tamin E levels to 1g total lipids was signifi-
cantly lower in a group of thalassemic pa-
tients, when compared to controls, indicating
true vitamin E deficiency (5). Therefore, one
may assume that decreased serum vitamin E
levels in thalassemia could be due to its in-
creased consumption for the neutralization of
free radicals and their deleterious effect on
RBC membranes. Since thalassemic RBC
membranes were found to have about twice
as much total phospholipids (4), the defi-
ciency of vitamin E could be even more
severe considering that lipids are the main
target of its action. Vitamin E deficiency in
thalassemia can eventually contribute to the
extent of the damage to the RBC and to other
tissues as well, as was suggested in cystic
fibrosis (6). For instance, it has been shown
in cystic fibrosis and also in normal controls
fed with vitamin E-deficient diet for 6 years
(24), that RBC survival is shorter than that
of normal RBC and could be corrected after
vitamin E administration. In a recent study
ineffective erythropoiesis and distinctive ul-
trastructural changes were found in normob-
lasts from vitamin E-deficient pigs, which
could be corrected after vitamin E adminis-
tration (25). Similar abnormalities are known
to exist in congenital dyserythropoietic ane-
mia and also in β-thalassemia major (26, 27).

A justified conclusion from all the afore-
mentioned observations was to start a therapeu-
tic trial of large doses of vitamin E in
patients with β-thalassemia major. The re-
results showed that it was possible to maintain
high serum levels and that one could double
or triple vitamin E levels in whole RBC or in
RBC membrane preparations, indicating that
the vitamin was able to reach at least one of
its main targets. Discontinuation of the vi-
tamin led to decrease in the serum levels to
the pretreatment values, further implying that
vitamin E deficiency in thalassemia is real
and severe and that there were no sufficient
stores to keep the excess vitamin for longer
periods of time.

The continuous increase in RBC and se-
rum vitamin E levels resulted in changes in
some biochemical parameters of the RBC. In
a previous study it was shown that in patients
with β-thalassemia who were treated with
vitamin E the increased resistance of RBC to
osmotic lysis could be corrected (12). The
results of the present work showed generation
of almost normal MDA levels in treated pa-
tients indicating that the RBC membrane
lipid is much less susceptible to exogenous
oxidative stress. The transfusion require-
ments did not show any significant changes between the treated and untreated patients. Similar, although preliminary and undetailed results have been reported by Modell et al. (28), RBC survival was prolonged only in three out of seven treated patients, but even in those patients there were no significant changes in the other hematological parameters. These results are different from those obtained in cystic fibrosis, where vitamin E administration resulted in prolongation of the relatively short RBC survival in all treated patients. A possible explanation for these results could be due to the fact that pathological changes occur not only in the thalassemic RBC membrane lipids, but in other components of the membrane as well: a significant decrease in titratable SH groups per milligram membrane protein, indicating protein oxidation, and in sialoglycoproteins and their distribution on the outer surface of the membrane (12, 29). Titratable SH groups in the treated patients did not change after vitamin E administration as shown in Table 1. Administration of vitamin E did not prevent cross-linkage of the membrane proteins with the same exogenous oxidative stress that was used to generate MDA (30). One can conclude that while there seem to be theoretical and practical indications to treat patients with β-thalassemia major and intermedia with vitamin E, it is incapable of correcting the variety of changes of all membrane components and one has to look for additional ways to complement the antioxidant properties of vitamin E, which is probably limited only to membrane lipid.

The authors thank Dr. Myron Brin and Dr. Paul Bermond of F. Hoffmann-La Roche & Co., Ltd., Nutley, N.J., and Basle, Switzerland, for their help and encouragement in performing these studies.

References