Failure of Vitamin E Therapy in the Treatment of Anemia of Protein-Calorie Malnutrition

By S. J. Baker, S. M. Pereira and Almas Begum

Vitamin E deficiency in monkeys has been described as causing an anemia characterised by multinucleated erythroid precursors in the bone marrow. Subsequently, it has been claimed that vitamin E is of therapeutic value in megaloblastic anemia of protein-calorie malnutrition and that the vitamin may play a role in human hemopoiesis. The present study was undertaken to determine the influence of vitamin E therapy on the hematologic status of South Indian children with protein-calorie malnutrition.

Material and Methods

Sixteen children aged 1 to 4 years, showing the edema, apathy and other associated features of protein-calorie malnutrition were included in the study. The children were admitted to the metabolic ward of the Nutrition Research Unit and preliminary hematologic data collected. They were maintained on a diet that provided 3 gms. protein and 150 calories/kg body weight/day from skimmed milk, sugar, rice and coconut oil. This diet provided approximately 3 mg alpha-tocopherol daily. A vitamin supplement was given, which did not contain vitamin B12 or folic acid.

Standard hematologic techniques as described by Dacie were employed. Hemoglobin was estimated by the cyanmethemoglobin method, using a photoelectric colorimeter calibrated and checked against a hemoglobin standard at regular intervals. Bone marrow smears, obtained by aspiration from the iliac crest, were stained with May-Grunwald-Giemsa stain. Megaloblastic marrows were graded I-IV according to severity, grade IV showing the most pronounced changes.

Serum vitamin B12 levels were estimated, using Euglena gracilis, Z strain, by the method of Hutner et al., and serum folate levels were measured using Lactobacillus casei as the test organism. Total serum proteins were determined by a microbiuret method. The protein fractions were obtained by paper electrophoresis at pH 8.6, staining with bromphenol blue and eluting the dye with 0.01 N sodium hydroxide.

A slight modification of the Emrie-Engel reaction described by Bieri et al. was used for the determination of alpha-tocopherol in the serum. The serum levels were estimated in eleven of the patients on admission to hospital, and in the other five before therapy with vitamin E was started. Serum levels were also estimated within 4 to 11 days after the first dose of vitamin E was given.
Table 1.—Hematologic Status of Children on Admission to Hospital

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age in months</th>
<th>Serum Albumin</th>
<th>Hb</th>
<th>Packed cell volume</th>
<th>Retics</th>
<th>Bone Marrow</th>
<th>Serum B&lt;sub&gt;12&lt;/sub&gt; μg./ml.</th>
<th>Serum Folate μg./ml.</th>
<th>On admission</th>
<th>Prior to Therapy</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>1.31</td>
<td>5.9</td>
<td>24</td>
<td>2</td>
<td>Megaloblastic III</td>
<td>480</td>
<td>11.2</td>
<td>656</td>
<td>557</td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>1.77</td>
<td>9.1</td>
<td>28</td>
<td>2.5</td>
<td>Megaloblastic II</td>
<td>620</td>
<td>8.0</td>
<td>—</td>
<td>600</td>
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<tr>
<td>3</td>
<td>41</td>
<td>2.07</td>
<td>10.8</td>
<td>30</td>
<td>2</td>
<td>Megaloblastic III</td>
<td>288</td>
<td>8.1</td>
<td>—</td>
<td>511</td>
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<tr>
<td>4</td>
<td>42</td>
<td>0.95</td>
<td>7.5</td>
<td>22</td>
<td>2</td>
<td>Megaloblastic I-II</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>44</td>
<td>1.39</td>
<td>7.7</td>
<td>26</td>
<td>4</td>
<td>Megaloblastic I</td>
<td>440</td>
<td>2.9</td>
<td>399</td>
<td>—</td>
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<tr>
<td>6</td>
<td>36</td>
<td>0.81</td>
<td>6.0</td>
<td>20</td>
<td>5</td>
<td>Normoblastic</td>
<td>1320</td>
<td>3.4</td>
<td>—</td>
<td>150</td>
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<tr>
<td>7</td>
<td>33</td>
<td>1.37</td>
<td>8.4</td>
<td>28</td>
<td>4</td>
<td>Megaloblastic I</td>
<td>108</td>
<td>14.0</td>
<td>95</td>
<td>125</td>
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<td>8</td>
<td>22</td>
<td>2.29</td>
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<td>27</td>
<td>1</td>
<td>Megaloblastic III</td>
<td>112</td>
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<td>300</td>
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<tr>
<td>9</td>
<td>13</td>
<td>1.77</td>
<td>10.2</td>
<td>30</td>
<td>2</td>
<td>Megaloblastic II</td>
<td>180</td>
<td>7.6</td>
<td>235</td>
<td>810</td>
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<tr>
<td>10</td>
<td>30</td>
<td>0.93</td>
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<td>3</td>
<td>Megaloblastic II</td>
<td>780</td>
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<td>1.11</td>
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<td>4</td>
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<td>264</td>
<td>6.0</td>
<td>—</td>
<td>602</td>
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<tr>
<td>12</td>
<td>17</td>
<td>1.25</td>
<td>6.1</td>
<td>19</td>
<td>1.5</td>
<td>Megaloblastic I</td>
<td>208</td>
<td>4.2</td>
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<td>525</td>
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<td>13</td>
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<td>1.08</td>
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<td>21</td>
<td>3</td>
<td>Megaloblastic I-II</td>
<td>460</td>
<td>6.4</td>
<td>300</td>
<td>495</td>
</tr>
<tr>
<td>14</td>
<td>34</td>
<td>2.12</td>
<td>11.6</td>
<td>33</td>
<td>2</td>
<td>Megaloblastic I-II</td>
<td>280</td>
<td>8.0</td>
<td>390</td>
<td>660</td>
</tr>
<tr>
<td>15</td>
<td>36</td>
<td>1.63</td>
<td>8.7</td>
<td>27</td>
<td>1</td>
<td>Normoblastic</td>
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<td>5.1</td>
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<td>16</td>
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<td>1.67</td>
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<td>30</td>
<td>2</td>
<td>Normoblastic</td>
<td>780</td>
<td>7.0</td>
<td>235</td>
<td>—</td>
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</table>
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After 1 to 2 weeks baseline observation, the children were given d, 1-α-tocopherol 100 mg. daily by intramuscular injection* and 100 mg. d, α-tocopheryl acetate as a water miscible preparation† orally, three times a day for 5 to 7 days.

Hemoglobin and packed cell volume estimations were done frequently on samples of venous blood and reticulocyte counts done more frequently, often daily, on capillary blood. Bone marrow estimations were done at approximately weekly intervals during the stay in hospital and one of these was always timed to be 7 to 10 days after the vitamin E was started.

After observing the response to vitamin E, children judged to be folate deficient were given a daily oral dose of 25 μg. of folic acid to observe the marrow response.

RESULTS

Status on Admission

The biochemical and hematologic status of the sixteen children on admission to hospital is presented in Table 1. All had evidence of moderate to severe kwashiorkor and all but three had a significant anemia. The average serum α-tocopherol of the 11 patients in whom values were estimated on admission was 369 μg. per cent with a range of 95-690 μg. per cent. In the other 5 subjects, serum α-tocopherol values were estimated before therapy with vitamin E was started, when the mean was 448 μg. per cent with a range of 150-602 μg. per cent.

In 7 subjects, serum α-tocopherol levels were estimated on admission and again before the start of vitamin E therapy. The values had increased in 5 of 7, with the average value rising from 415 to 580 μg. per cent.

At the time of admission, 3 children had a normoblastic bone marrow and 13 had a megaloblastic marrow.

Serum vitamin B12 and folate levels showed a wide scatter. In 6 cases, apparently normal serum vitamin B12 and folate levels were found in children who had megaloblastic changes in the bone marrow, as previously described.17 In 5 of these the serum folate and in 1 the serum vitamin B12 fell to below normal levels during their stay in hospital.

Response to Vitamin E Therapy

On treatment with vitamin E, 8 subjects, 2 with normoblastic and 6 with megaloblastic marrows, showed no hematologic response, i.e., no change in reticulocyte count, hemoglobin or packed cell volume. Bone marrow morphology remained the same in 5 subjects (4 megaloblastic, 1 normoblastic). In 2 subjects the megaloblastic changes increased (grade I to grade II and grade II to grade III) and in 1 subject the normoblastic bone marrow changed to grade I megaloblastic.

Two children with megaloblastic bone marrows showed a rise in reticulocyte count 13 and 14 days after the start of therapy with no significant change in hemoglobin or packed cell volume. In one of these there was no change in the bone marrow morphology, whereas in the other the degree of megaloblastosis increased from grade I to grade II.

*Epsilin oil, Warren-Teed Products Co., Columbus 15, Ohio.
†Aquasol E, U.S. Vitamin & Pharmaceutical Corp., New York, N. Y.
Table 2.—**Packed Cell Volumes, Reticulocyte Responses, Bone Marrow Morphology and Serum Vitamin E Values Before and 8-10 Days After Vitamin E Therapy in the Six Subjects who Showed a Reticulocytosis**

<table>
<thead>
<tr>
<th>Case number</th>
<th>Packed Cell Volume</th>
<th>Day of Maximal Reticulocyte Response</th>
<th>Bone Marrow</th>
<th>Serum Vitamin E μg. %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>1</td>
<td>24</td>
<td>25</td>
<td>9%</td>
<td>8th day</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>30</td>
<td>18%</td>
<td>5th day</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>29</td>
<td>16%</td>
<td>7th day</td>
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<tr>
<td>4</td>
<td>28</td>
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<td>20%</td>
<td>8th day</td>
</tr>
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<td>5</td>
<td>28</td>
<td>29</td>
<td>14%</td>
<td>7th day</td>
</tr>
<tr>
<td>6</td>
<td>18</td>
<td>20</td>
<td>25%</td>
<td>8th day</td>
</tr>
</tbody>
</table>

*Serum value on admission.
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Case no. 2

Fig. 1.—The hematologic response of a child with protein-calorie malnutrition to vitamin E, iron and folic acid.

Six children (5 with a megaloblastic and 1 with a normoblastic bone marrow) had a rise in the reticulocyte count following vitamin E therapy with the peak on the fifth and eighth day after starting therapy and in five of these there was a slight but not continuing rise in packed cell volume (Table 2). None of the five subjects who initially had a megaloblastic bone marrow showed any change in marrow morphology when reexamined 7 to 10 days after the start of therapy. The child with the normoblastic bone marrow at the start of therapy, had grade I megaloblastic changes when the marrow was repeated on the tenth day after commencement of therapy. Figure 1 shows the hematologic response in case number 2. It will be seen that the reticulocyte count following vitamin E therapy was not accompanied by any change in marrow morphology. A second reticulocyte response followed the administration of iron and the marrow reverted to normal after folic acid therapy.

Thirteen of the total of 16 patients were subsequently treated with hematicins: iron (3), folic acid (9) and vitamin B₁₂ (1). Two of these children were discharged to their homes before a hematologic response could be recorded. In the other children with a megaloblastic marrow, there was a reticulocytosis and a reversion of the marrow to a normoblastic pattern of erythropoesis following folic acid or vitamin B₁₂.

DISCUSSION

The hematologic status of the subjects included in the study and the diet they were fed were identical with that of the children with protein-calorie malnutrition reported previously from this center. The diet provided approx-
approximately 3 mg. of α-tocopherol daily which tended to produce a rise in serum levels during the period of observation (Table 1). Similar rises in serum levels from this amount of dietary α-tocopherol have been reported in other subjects. In our experience, this diet does not produce hematologic responses and there is in fact a tendency for the anemia to increase.

Reports in the literature on the role of vitamin E deficiency in relation to anemia in man are conflicting. The association of low serum tocopherol levels with increased peroxide hemolysis in vitro has been well documented. A very slight decrease in "total" 51Cr red cell survival in subjects with low serum tocopherol and increased peroxide hemolysis was reported by Horwitt et al., but none of the subjects was anemic. On the other hand, normal red cell survival was recorded by Asfour et al. in children with low serum tocopherol values. Red cell peroxide hemolysis was not investigated in the present study, since, apart from being a rather indirect and slightly unreliable indicator of plasma vitamin E levels, the results of the test have not been shown to have clinical significance.

Goldbloom maintained premature infants on tocopherol free diets. The infants developed extremely low serum vitamin E levels but did not become anemic and had hemoglobin and hematocrit values comparable with infants on "normal" and vitamin E fortified formulas. Oski and Barness found that the administration of vitamin E to premature infants with low serum tocopherol levels between six and eleven weeks of age caused a significant rise in hemoglobin. However, in a subsequent study they were unable to duplicate these results. In a careful study in premature infants by Panos et al. it was concluded that the anemia of prematurity was not related to vitamin E deficiency. The adult subjects of Horwitt et al. on an experimental E deficient diet for over six years did not become anemic, and subjects with acanthocytosis and no detectable vitamin E in their plasma do not generally have significant anemia.

Apart from the studies in patients with protein-calorie malnutrition there is therefore no evidence to suggest that vitamin E deficiency per se causes anemia or that it has any role in human hemopoiesis.

In cases of protein-calorie malnutrition it has been claimed that treatment with vitamin E "induces a favorable hematologic response in malnourished infants with macrocytic anemia" and "a consistent hematologic response to vitamin E" was obtained. On the other hand, Asfour et al. were unable to demonstrate that vitamin E had any hematologic effect on anemic undernourished children with very low serum α-tocopherol levels. The claims for the therapeutic effect of the vitamin are based specifically on a study of patients with protein-calorie malnutrition, and it is possible that vitamin E therapy might have an effect in this condition which was not seen in the cases of undernutrition studied by Asfour.

The serum α-tocopherol levels of the children in this study are, in general, comparable with those reported in previous studies from other centers. However, no consistent hematologic response to vitamin E therapy was observed in these children. In 8 cases there was no detectable change in hema-
tologic status, except that in 3 the marrow abnormalities became more pronounced. In 2 cases peak reticulocyte responses were obtained on the thirteenth and fourteenth days, respectively, after the beginning of vitamin E therapy. These cannot therefore be considered as occurring within the acceptable limits of a reticulocyte response to a hematinic. Such apparently spontaneous reticulocyte responses have previously been seen occasionally in our patients not given vitamin E. Only 6 out of the 16 children studied showed a reticulocyte response which could temporally relate to vitamin E therapy. In all these cases, however, there was not the expected sustained increase in hemoglobin and packed cell volume, such as usually follow a reticulocyte response to a true hematinic and in none of the 6 was there improvement in marrow morphology. It might be contended that therapy was only given for five days, but vitamin E levels were found to be well above normal values even two weeks after therapy.

Inspection of the "typical example of the hemoglobin and reticulocyte response to vitamin E therapy" (patient 1) of Whitaker et al. shows a rise in hemoglobin of approximately 4 Gm. per cent occurring in two days time and preceding the rise in reticulocytes by 3 days. Such a precipitous rise in hemoglobin can apparently only be explained by loss of plasma water and cannot represent a hematologic response to vitamin E therapy. Further, a number of the reticulocyte responses reported from Thailand and Jordan are hematologically inadequate or temporally unacceptable as a response to a hematinic.

The so called "hematologic responses" following vitamin E therapy obtained by us and other workers are reminiscent of the nonspecific type of response previously recorded in patients with pernicious anemia treated with arsenic. From the present study and other studies so far published, there is little or no indication that vitamin E plays a significant role in human hemopoiesis. On the evidence currently available, with the possible exception of premature infants, it appears that a low vitamin E level in human subjects is still a biochemical finding in search of a disease.

**Summary**

A study is reported of the hematologic changes occurring in 16 children with protein-calorie malnutrition treated with vitamin E. Eight children showed no response. Two showed a very delayed rise in reticulocyte count and six had a peak reticulocyte response 5 to 8 days after starting therapy. None of the patients showing reticulocyte responses had a sustained rise in hemoglobin or packed cell volume. In none of the cases did the marrow become normoblastic following therapy and in five the marrow abnormalities became more marked.

The results of this study do not confirm the suggestion that vitamin E has a significant role in human hemopoiesis.

**SUMMARIO IN INTERLINGUA**

Es reportate un studio del alterationes hematologic occurrente in 16 juveniles con malnutrition proteinic e caloric qui esseva tractate con vitamina E. Octo del subjectos
monstrava nulle responsa. Duo monstrava un retardatissime augmento in le numeration del reticulocytos, durante que 6 hebaeva un maxime responsa reticulocytic inter 5 e 8 dies post le institucion del therapia. Nulle del patientes con positive responsas reticulocytic manifestava un persistente augmento del volumines de hemoglobin a o de cellulas pacate.

In nulle del casos deveniva le medulla normoblastic post le therapia e in cinque le anormalitates medullari deveniva plus marcate.

Le resultatos de iste studio non confirma le suggestion que vitamina E ha un rolo significative in le hematopoiese human.

REFERENCES

VITAMIN E THERAPY

Failure of Vitamin E Therapy in the Treatment of Anemia of Protein-Calorie Malnutrition

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