Vitamin E Responsive Megaloblastic Anemia in Infants with Protein-Calorie Malnutrition

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THE predominant manifestations of vitamin E deficiency in nonprimate species are nutritional muscular dystrophy and reproductive difficulties.[1-3] In the monkey a deficiency of vitamin E produces anemia,[4,5] and it has been suggested[4] that some aspects of anemia in man might be the counterpart of this experimental syndrome. One of us (A.S.M.) repeatedly has called attention to a macrocytic anemia present in infants in Jordan[6,7,23] which did not consistently respond to treatment with vitamin B_{12}, folic acid, ascorbic acid or iron. This present report describes the response of patients with this anemia to treatment with vitamin E.

EXPERIMENTAL

Studies were conducted in the Pediatrics Department of the Augusta Victoria Hospital, Jerusalem, Jordan. The subjects were twelve infants of both sexes six months to two years of age. All of them were underweight and exhibited growth retardation, muscular wasting, and dyspigmentation or had hair that was easily pulled out as well as other signs characteristic of protein-calorie malnutrition. Seven had marasmus and five had kwashiorkor with edema and hypoproteinemia. Some of the infants were severely dehydrated, and the degree of the anemia was not fully apparent until they had been rehydrated during a few days of hospitalization. Regardless of the variation in other aspects of the clinical picture the anemia was of the same type in each subject and varied only in degree.

All the infants were being or had been breast fed. The usual history was that of a healthy infant until some five months or more of age at which time there was onset of the familiar sequences of failure to gain in weight, febrile illness or diarrheal disease and recent development of edema or some other evidence of illness alarming to the mother. It is pertinent that in the present series most of the infants were admitted during the concluding phase of the diarrheal season (August to November), although the initial infant in this series was observed in June. Frequently the mother had instituted unsuitable changes in the infant's feeding, such as restricting him to a diet of rice water or other gruel, dilute condensed milk and tea. The majority of patients were the offspring of mothers who for years had subsisted on limited diets which were based on rations[8] consisting of white flour, pulses, rice, sugar, dried fruit and fat. This supplies 1,500 to 1,600 calories daily per capita. The protein of the basic ration upon calculation is relatively low in methionine. Supplements of skim milk powder are available for children and pregnant and lactating women.

Twelve infants exhibiting anemia associated with evidences of protein-calorie malnutrition were selected. Upon admission they were placed on a regimen of prophylactic penicillin and streptomycin and, if they were severely dehydrated, a routine to re-establish electrolyte and water equilibrium was initiated. Most of the infants were given a skimmed

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This study was supported in part by U. S. Public Health Service Grant A-5499 NTN and ONR Contract No. Nonr 2149(04).

milk-casein formula, but some initially received a whole milk diet. Supplements of B vitamins were given to some, but no iron, vitamin B_{12} or folic acid was administered. Although complete dietary control of the infants was not always possible, the degree of control maintained was adequate. In all instances a period of not less than two to four weeks of daily observation in the hospital elapsed before specific treatment was instituted. The consistent pattern of timing of the responses provided convincing evidence of the relationship of the specific therapy and the hematologic response.

After stabilization of the patient, daily reticulocyte counts were made with frequent determinations of erythrocytes, hematocrit and hemoglobin. Bone marrow examinations were made immediately prior to the institution of specific treatment and after a week or more of therapy. A sample of urine was collected daily for determination of creatine:creatinine ratio[4] before and after treatment. Plasma vitamin E[9] and serum vitamin B_{12} levels were measured prior to therapy in most cases.

During the two to four week initial control period in the hospital the children usually were maintained on the skim milk-casein enriched diet.* During the first or second week of this regimen a transitory increase in reticulocytes was sometimes observed, but this was not followed by significant hemopoiesis prior to the institution of specific therapy. The bone marrow examinations carried out before treatment were made after this initial increase in reticulocytes and revealed that the maturation defect persisted until institution of specific therapy. The cause of this initial increase in reticulocytes is under further investigation.

After the preliminary observation period and after any initial increase in reticulocyte count had subsided, treatment was started with vitamin E; 100 mg. of alpha tocopherol phosphate† was given intramuscularly and 280 mg. of alpha tocopherol acetate† orally per day for five days. The daily observations of reticulocyte count, urine collections and frequent blood counts were continued. A second blood sample was obtained for determination of serum tocopherol and vitamin B_{12} levels approximately three weeks after treatment was discontinued. Bone marrow specimens were again taken six days or more after therapy was initiated.

* This formula consists of 50 gm. skimmed milk powder, 50 gm. calcium caseinate (Casilan,® Galaxo), 20 gm. sugar and 1 L. water.

† Nutritional Biochemicals, Inc.

RESULTS AND COMMENTS

An example of the hematologic response of an anemic child to vitamin E therapy is shown in Figure 1. In this patient the peak reticulocyte response occurred on the sixth day of treatment and was followed by a sustained increase in erythrocyte count, hemoglobin and hematocrit. The relationship of the hematologic response and the degree of anemia is shown in Figure 2 for the eight subjects in whom full hematologic data were determined. The weekly increase in erythrocyte count was calculated for the first two to three weeks after treatment. A linear relationship exists between the severity of the anemia and the magnitude of the reticulocyte response and erythrocyte increase. The quantitative nature of these responses to tocopherol agrees with that expected in patients with well treated pernicious anemia.[10]

Erythrocyte values before and after treatment with vitamin E are given in Table 1. The anemia was macrocytic. Vitamin E treatment resulted in reduced mean cell volume and mean cell hemoglobin, but did not alter the mean cell hemoglobin concentration.

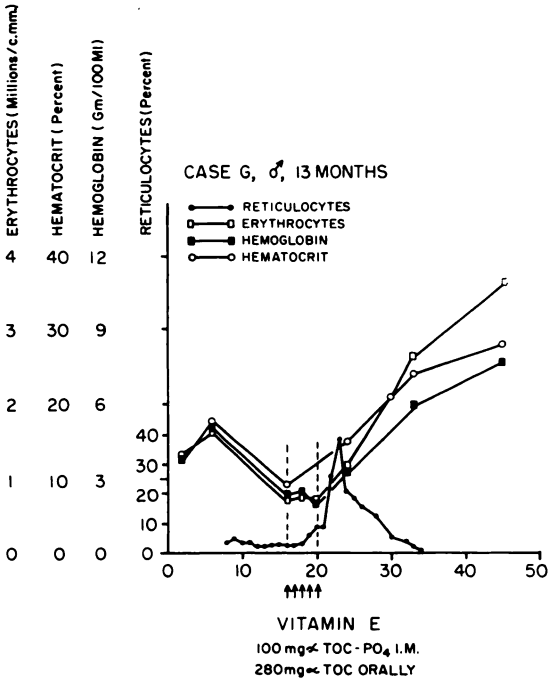

FIG. 1. Hematologic response of an anemic infant to treatment with alpha tocopherol.

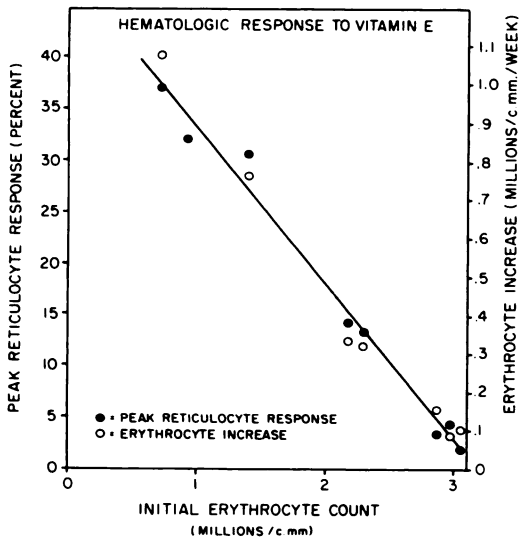

FIG. 2. Relationship of hematologic response following treatment with vitamin E to severity of anemia. Erythrocyte data omitted on one patient because of complicating transfusion after reticulocyte peak attained.

Prior to vitamin E therapy all bone marrow specimens examined showed changes associated with a megaloblastic type marrow. These included changes in the red blood cell as well as in the white blood cell precursors and were irrespective of diet, duration in hospital or modalities of therapy other than vitamin E.

The bone marrow specimens were hypercellular with respect to the three formed elements, and mitotic figures of erythroid elements were frequent. Some binucleated megaloblasts as well as binucleated normoblasts, similar to those reported by Porter et al.[5] as occurring in vitamin E-deficient monkeys, were seen.

The peripheral smears showed anisocytosis and poikilocytosis and marked macrocytosis with characteristic macro-ovalocytes, multilobed polymorphonuclear leukocytes and giant platelets. There were many target cells, pencil and cigar forms that are usually seen with hemoglobin synthesis defects; these may reflect a coexisting iron and/or pyridoxine deficiency. Megaloblasts were seen in the peripheral smears of the more anemic patients.

All these findings in the marrow specimens and peripheral smears reverted to normal following therapy with vitamin E. The megaloblastic erythropoiesis became normoblastic within six days after the start of therapy. This reversion may have occurred earlier but bone marrow studies were not made sooner after treatment. The macrocythemia often was reversed to microcythemia following specific therapy. The reticulocyte response following vitamin E therapy was brisk; the peak occurred in three to fourteen days from the onset of treatment and was shortly followed by a commensurate increase in red blood cell count, hematocrit and hemoglobin.

According to all available data this anemia resembles and behaves like the megaloblastic anemia of vitamin B_{12} or folic acid deficiency, i.e., it is an anemia of "maturation arrest" due to a deficiency of a factor essential to hematopoiesis.

Prior to treatment with vitamin E, serum vitamin B_{12} levels were determined in nine patients employing Euglena gracilis "L-strain." The values were as follows: 343, 736, 170, 948, 46, 64, 379, 177 and 512 $\mu\mu$g. per ml. It was of interest that the two patients with initial erythrocyte counts of less than 750,000 exhibited serum vitamin B_{12} levels of 948 and 46 $\mu\mu$g., respectively. Both gave maximum hematologic responses to vitamin E therapy without any administration of vitamin B_{12}.

Creatine excretion is increased in tocopherol-deficient animals, and the reduction of the creatine:creatinine ratio is a most sensitive index of recovery. From the results presented

TABLE I
Erythrocyte Values Before and After Treatment with Vitamin E*

Time of Test	Mean Corpuscular Volume (MCV)	Mean Corpuscular Hemoglobin (MCH)	Mean Corpuscular Hemoglobin Concentration (%) (MCHC)
Before treatment	112 ± 5	32 ± 0.6	29 ± 2.6
After treatment	91 ± 5	25 ± 1.1	28 ± 2.2

* Eight cases; values are ± mean standard error.

in Figure 3 it may be seen that the urinary creatine:creatinine ratios of these anemic children were elevated before treatment and were reduced to normal levels during treatment with vitamin E. Normal levels were established by determining the urinary creatine:creatinine ratios in fourteen children aged six months to two years in a local well baby clinic. It is to be noted that the malnourished anemic infants exhibited pronounced muscular wasting and were recovering from severe malnutrition. Since these factors might have been expected to favor creatinuria, the observed reduction appears to be even more meaningful.

The mean plasma tocopherol concentration determined in eighteen anemic children was 0.48 mg. per 100 ml. with a standard error of ±0.04. The values ranged from less than 0.1 to 0.7 mg. The serum tocopherol levels in six infants before and after treatment are given in Table II. The tocopherol procedure measures both tocopherol and ubiquinone, hence the actual blood levels of vitamin E are probably lower than reported. In our laboratory the mean serum tocopherol value for eleven normal adults was 1.16 mg. per 100 ml. with a standard error of ±0.09. Serum vitamin E levels were lower in the anemic children and, while the levels after treatment were higher, normal adult levels still were not attained. This suggests that the total quantity

TABLE II

Plasma Tocopherol Levels in Anemic Children Before and After Treatment with Alpha Tocopherol*

Case No.	Before Treatment	After Treatment†
1	0.45	0.59
2	0.33	0.59
3	0.45	0.65
4	0.37	0.93
5	0.35	0.73
6	0.65	0.89
Average	0.43	0.73

* Values are expressed as milligrams tocopherol per 100 milliliters plasma.

† Values obtained after treatment were determined three weeks after vitamin E therapy was discontinued.

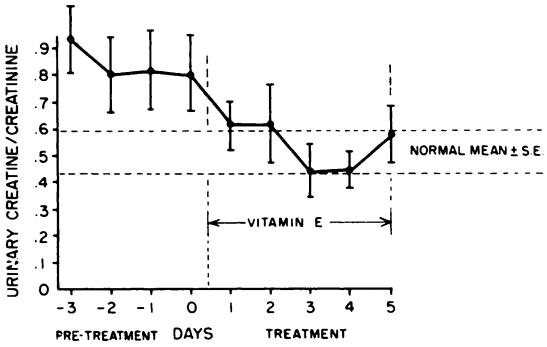

FIG. 3. Influence of vitamin E therapy on urinary creatine:creatinine ratios of infants, average of eight cases. The normal values are the means ± standard error of fourteen infants in a well baby clinic.

of vitamin E administered may have been insufficient for complete repletion.

These tocopherol levels are not as strikingly low as those which have been observed in patients with sprue,[11] premature or newborn infants or those with steatorrhea,[12] an adult with tocopherol deficiency[13] or adults with Horwitt's induced vitamin E deficiency.[14] The average values before treatment are similar to those reported by Scrimshaw et al.[15] in patients with kwashiorkor, but lower than those reported in Central American school children[16] or iron-deficient infants in Nashville, Tennessee.[17] The findings suggest that, under certain dietary and metabolic conditions, the serum tocopherol concentrations do not have to be reduced dramatically in order to obtain a response to therapy. Final interpretation of the significance of given serum levels of tocopherol in such malnourished infants must await better understanding of the metabolic interrelationships involved. It is known that diarrhea in infants may deplete the content of fat-soluble vitamin K[18] and that fat-soluble vitamin levels in the serum may be altered by protein nutriture.[15,20]

The detailed data on another patient are given in Figure 4 and illustrate the correlation of responses described. The serum tocopherol and vitamin B_{12} values before treatment were low. The bone marrow was megaloblastic, and urinary creatine:creatinine ratios were elevated. Treatment with vitamin E induced a reticulocytosis, and the bone marrow reverted to normal. This was accompanied

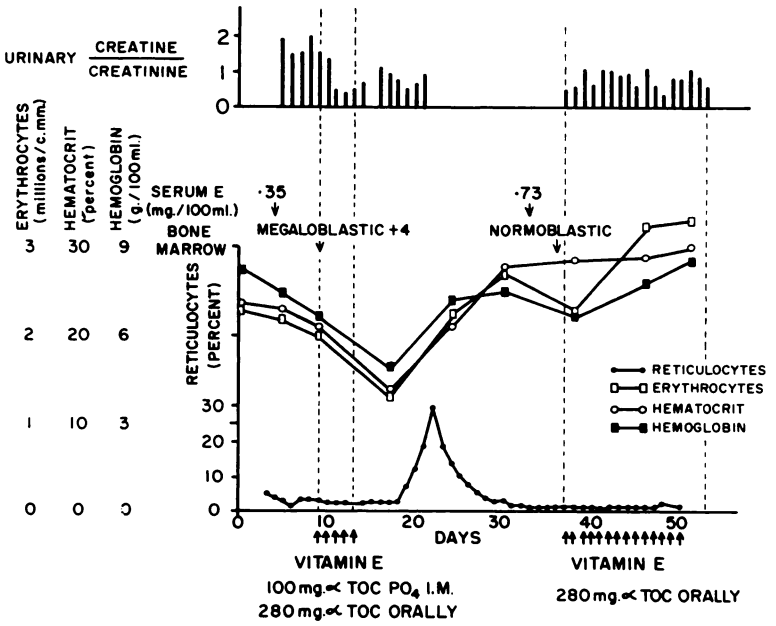

FIG. 4. Response of an infant to therapy with vitamin E.

by an increase in peripheral erythrocytes. Serum vitamin B_{12} and E levels increased after treatment. Creatine:creatinine ratios were reduced promptly when tocopherol was given. Creatine excretion tended to increase after discontinuation of treatment. This phenomenon was seen in all patients and is an indication that the dosage of vitamin E was inadequate for repletion.

The response of patients with this anemia to vitamin E therapy is similar to the response of others with macrocytic anemias to treatment with folic acid and vitamin B_{12} and suggests that these three vitamins may function in some closely related metabolic events. There is evidence that these vitamins influence deoxyribonucleic acid metabolism[19] which, in turn, may explain their effect on bone marrow maturation.

These experiments demonstrate that vitamin E now may be considered a hematopoietic agent in man as well as in monkeys. The factors leading to a deficiency of vitamin E in these children remain to be determined. Vitamin E nutriture of the infants may have been lowered because of preceding dietary, decreased absorption[18,20] due to the gastroenteritis, transport or metabolic changes resulting from the protein-calorie malnutrition or low reserves at birth due to maternal deficiencies. The inverse relationship between the intake of the sulfur amino acids and vitamin E requirement[21] is of interest.

The reponse of this series of patients to tocopherol therapy does not preclude the possibility that a similar response might have occurred in certain infants to other hematopoietic factors—vitamin B_{12}, folic acid or vitamin C. The variable responsiveness of infants with megaloblastic anemias[24] as well as those in other age groups is documented abundantly. Indeed, the same person frequently has been observed[22] to respond to either vitamin B_{12} or folic acid therapy in successive relapses.

SUMMARY

Treatment with vitamin E has been shown to induce a favorable hematologic response in malnourished infants with macrocytic anemia. These changes include typical therapeutic-induced increase in reticulocytes, followed by erythropoiesis and abatement of macrocythemia and conversion of megaloblastic marrow to a normoblastic one. There was a restoration to normal of urinary creatine:

creatinine ratio and an increase in previously low serum vitamin E levels.

These findings suggest that this anemia may represent the counterpart in man of the anemia which occurs in tocopherol-deficient monkeys. They provide further support to the concept of closely interrelated metabolic functions of tocopherol, vitamin B_{12} and folic acid.

It seems reasonable to believe that similar cases may be found elsewhere in regions in which protein-calorie malnutrition is common. The possibility of tocopherol deficiency may deserve further nutritional consideration than it has received in the past.

ACKNOWLEDGMENT

We are grateful to Miss Aida Nureddin, B.S., for technical assistance in the biochemical laboratory.

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