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The Occurrence and Effects of Human Vitamin E Deficiency: *A STUDY IN PATIENTS WITH CYSTIC FIBROSIS*

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ABSTRACT The role of vitamin E in human nutrition was studied by investigation of patients with cystic fibrosis (CF) and associated pancreatic insufficiency. Vitamin E status was assessed by measurement of the plasma concentration of the principal circulating isomer, α-tocopherol. Results of such determinations in 52 CF patients with pancreatogenic steatorrhea revealed that all were deficient in the vitamin. The extent of decreased plasma tocopherol varied markedly but correlated with indices of intestinal malabsorption, such as the serum carotene concentration and percentage of dietary fat absorbed. Supplementation with 5–10 times the recommended daily allowance of vitamin E in a water-miscible form increased the plasma α-tocopherol concentrations to normal in all 19 CF patients so evaluated.

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It is concluded that CF is invariably associated with vitamin E deficiency, provided that the patient in question has pancreatic achylia and is not taking supplementary doses of tocopherol. Concomitant hematologic effects consistent with mild hemolysis, but not anemia, occur and may be reversed with vitamin E therapy. Patients with CF should be given daily doses of a water-miscible form of vitamin E to correct the deficiency.

INTRODUCTION

A variety of vitamin E deficiency syndromes are readily produced in lower animals, but man (with the possible exception of the premature infant) has not been shown to develop symptoms in the face of diminished tocopherol. The usual dietary intake of tocopherol in the United States is adequate to maintain normal plasma levels in children and adults with unimpaired gastrointestinal function. On the other hand, biochemical evidence of vitamin E deficiency has been found in patients with malabsorption of

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various etiologies (1, 2) and in prematurely delivered newborns (3, 4). To study chronic vitamin E deficiency in children and adults, it is therefore necessary to utilize patients with intestinal malabsorption. Despite the presence of abnormalities in some laboratory indices identified in earlier work (1, 3), there are no convincing data that define the physiological derangements attributable to low tocopherol in these patients (5).

Of several disorders accompanied by steatorrhea, cystic fibrosis (CF)[1] with pancreatic achylia represents one of the most common causes of the malabsorption syndrome in the United States (6). Previous studies on the vitamin E status of CF patients, however, have been limited in scope and hampered by the small population of available subjects (1, 7). This work has also yielded conflicting data in regard to both the concentration of circulating tocopherol in malabsorption states and the susceptibility of erythrocytes to peroxide-induced hemolysis (an index of erythrocyte vitamin E) (1, 8, 9). Another limitation of previous studies is that they have not provided data on the ratio of α-tocopherol to circulating lipid, an expression which represents the most reliable index of vitamin E status routinely available in humans (10).

The existence of the above discrepancies and the complex presentation of tocopherol deficiency syndromes in animals indicated to us that a comprehensive approach with a large population of patients was most desirable to study vitamin E deficiency in CF. Accordingly, a group of such patients was evaluated with the overall goal being to determine whether or not vitamin E deficiency in man, generally, and in CF patients, particularly, leads to significant disturbances such as those which have been identified in animals. Specifically, the degree of vitamin E deficiency, its relationship to the extent of fat malabsorption, and the possible effects of diminished tocopherol were examined in these patients.

METHODS

Subjects. A total of 61 CF patients, 25 females and 36 males, were available for evaluation. The ages of these patients ranged from 1 to 42 yr (mean = 17 yr). Their diagnoses were established firmly by the presence of at least three of the four standard criteria for the disease: positive family history, pancreatic insufficiency, chronic obstructive pulmonary disease, and elevated sweat electrolytes. For purposes of this investigation, the patients were divided into three groups. The first group consisted of 52 subjects who had clinical or laboratory evidence of pancreatic insufficiency and who were not receiving supplementary tocopherol when the study began. The second group was comprised of seven patients with pancreatic insufficiency who, when first evaluated, were consuming 1–10 IU/kg per day of vitamin E (either on a referring physician's instructions or on a self-prescribed basis). The third group consisted of two patients who were not ingesting supplementary vitamin E, but who had evidence of either partially or completely intact digestive function.

The control subjects were 32 young adults ranging from 18 to 40 yr of age. These were either normal volunteers or employees of the National Institutes of Health. None had any history or evidence of pulmonary or gastrointestinal disease.

Samples obtained for analysis. Informed consent was obtained from all patients and control subjects used in the procedures described herein. Blood samples, obtained in the fasting state by venipuncture, were heparinized, centrifuged within 2 h, and the plasma collected and frozen for 1–5 days before extraction of lipids. Erythrocytes utilized for measurement of α-tocopherol concentration were washed twice with phosphate-buffered saline (PBS) at pH 7.4. Tissues were available from four patients who expired during the course of these studies and came to autopsy within 12 h of death. Whenever possible, 1–5-g samples of liver, heart, psoas muscle, lung, and adipose tissue were obtained, removed of adhering fat, and frozen at −20°C until tocopherol determinations could be performed (generally within 1 wk).

Determination of α-tocopherol. Measurement of α-tocopherol, the most abundant and active isomer of vitamin E in man, was performed after thin-layer chromatography by the method of Bieri and Prival (11) with minor modifications. For analyzing plasma, hexane extracts were concentrated and applied in bands to 20 × 20-cm plates containing a 250-μm coating of fluorescein-impregnated silica gel G. After development in benzene-ethanol (99:1), the α-tocopherol bands were removed and quantitated spectrophotometrically by reaction with $FeCl_3$ and bathophenantholine (12). Recovery studies were carried out at monthly intervals with 1 μg of α-tocopherol added per sample; for 20 samples analyzed, a mean±SE recovery of 80±2.8% was obtained. Results were therefore routinely corrected for a 20% loss during extraction and analysis.

Erythrocyte and tissue α-tocopherol was measured after saponification under nitrogen in the presence of pyrogallol as described by Bieri (12). The type of thin-layer plate and the first solvent were the same as for plasma analysis; the second solvent was hexane:ethanol (9:1). Trace amounts of ^{3}H-α-tocopherol were added in the initial extraction step for each sample, recoveries determined, and final results correlated for losses during analysis as described previously (13).

Erythrocyte hemolysis in vitro. This was measured after addition of hydrogen peroxide (H_2O_2) with a technique modified from that described by Horwitt et al. (14). In this procedure, erythrocytes were processed within 2 h of collection by washing twice with PBS at 37°C and by preparing a final 5% suspension in the same solution. Aliquots of 0.3 ml each were then pipetted into seven disposable plastic tubes (12 × 75 mm) to conduct the hemolysis test per se. Preliminary studies were carried out to establish optimal conditions, and the following approach was adopted for routine use: (*a*) 0.3 ml of 4% hydrogen peroxide, freshly diluted in PBS, was added with a plastic pipette to the first three tubes containing erythrocyte suspensions (in pipetting H_2O_2, the solution was not allowed to contact the walls of the test tube before entering the suspension of cells); (*b*) 0.3 ml of PBS was added to the fourth and fifth tubes; and (*c*) distilled water in a volume of 3.8 ml was added to the last two samples. All tubes were then covered with parafilm, gently mixed by two inversions, and incubated at 37°C for 3 h.

[1] *Abbreviations used in this paper*: CF, cystic fibrosis; PBS, phosphate-buffered saline; RBC, erythrocytes.

TABLE I
Vitamin E and Triglyceride Levels in Blood from CF Patients with Pancreatic Insufficiency

	Control	CF
Plasma α-tocopherol, *μg/dl*	683±33 (n = 32)	107±13* (n = 52)
Plasma triglycerides, *mg/dl*	106±7.9 (n = 32)	120±9.4 (n = 20)

* $P < 0.001$.

Tubes 1–5 were then diluted to a final volume of 4.1 ml with PBS. All samples were centrifuged, and the absorbance of the supernate was measured at 575 nm as an indication of the degree of hemolysis. Results are expressed as the percent of total hemoglobin released from cell suspensions which received distilled water.

Erythrocyte survival. This was assessed by measuring the half-life of ^{51}Cr-labeled, autologous erythrocytes. The specific procedure employed was Method C recommended by the International Committee for Standardization in Hematology (15). Labeling with radiochromium was carried out with 10-ml samples of venous blood mixed with acid-citrate dextrose; approximately 1 μCi of ^{51}Cr was added per kilogram of body weight. Blood samples of 5-ml volume were drawn 1 h after injection and every 2 or 3 days until a total of 12 specimens were available for determination of radioactivity. All tubes were counted at the same time to a statistical accuracy of ±2%. Data obtained were corrected for chromium elution, which was found to be the same for vitamin E-deficient erythrocytes and control samples.

Absorptive function studies. These were carried out on 21 patients admitted to the National Institutes of Health Clinical Center for at least 1 wk who cooperated for balance studies. They were fed a diet of known composition providing approximately 100 g of fat per day. A carmine marker was given on the first hospital day and when this had been excreted, stool collection began and continued for 72 h. Pools of feces were then weighed and homogenized, and duplicate aliquots removed for extraction and measurement of fat as total fatty acids by the method of van de Kamer et al. (16). The concentration of carotene in serum samples from 25 CF patients was determined by extraction with petroleum ether and measurement of absorbance at 450 nm.

Lipid determinations. The triglyceride concentration of plasma samples obtained in the fasting state was measured by the procedure of Kessler and Lederer (17). Total plasma lipids were measured by the turbidimetric method of de la Huerga et al. (18) after extraction with ethyl ether/ethanol (1:3). The fatty acid composition of erythrocytes was determined with a total lipid extract prepared with isopropanol-chloroform according to the procedure described by Bieri and Poukka (19).

RESULTS

Vitamin E status of CF patients before supplementation. As indicated in Table I, the 52 CF patients with evidence of steatorrhea exhibited a significant decrease in the mean level of α-tocopherol, with an average value equal to only 15% of that found in 32 control subjects. Plasma triglyceride concentrations, measured in 20 young adults with CF, were not found to be statistically different from values obtained in age-matched controls. Thus, the ratio of α-tocopherol to triglycerides in plasma was markedly lower in CF patients (0.89 μg/mg), compared to normal subjects (6.44 μg/mg). A similar difference was noted in comparing α-tocopherol/total lipid ratios in a limited number of subjects. Five CF patients showed a total lipid concentration of 385±23 mg/dl (mean±SE) and a ratio of 0.42±0.19 μg α-tocopherol per mg lipid; corresponding values in five controls were 545±18 mg/dl (P

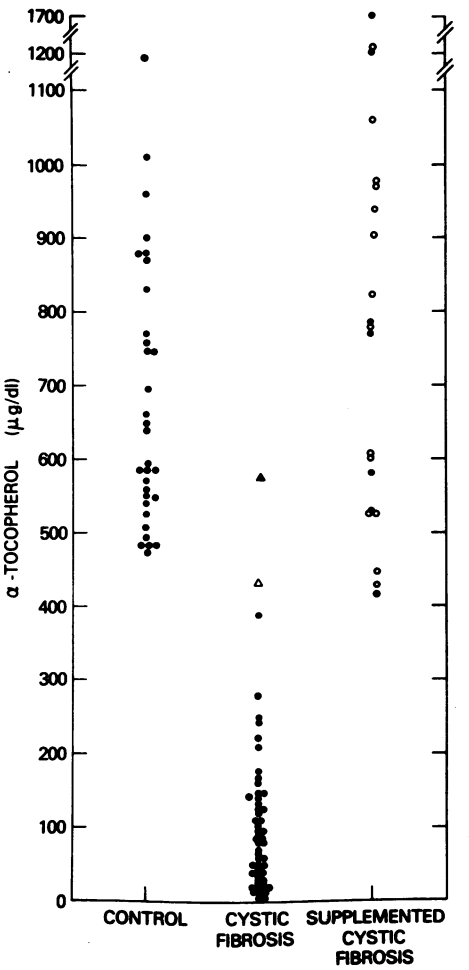

FIGURE 1 Plasma α-tocopherol concentrations in control subjects and CF patients. All of the latter group had evidence of steatorrhea except for the patient indicated by the filled triangle who showed normal pancreatic enzyme activities on assay of duodenal contents and 99% absorption of dietary fat. The CF patient indicated by the open triangle had border-line fecal fat excretion but deficient pancreatic enzymes on duodenal drainage. Vitamin E-supplemented patients were either receiving α-tocopheryl acetate when these studies commenced (closed circles) or were placed on the vitamin after a period of evaluation in the basal state.

< 0.001) and 1.18 ± 0.05 μg tocopherol per mg lipid ($P < 0.005$).

Individual values of plasma α-tocopherol are given in Fig. 1 for controls, CF patients with or without pancreatic achylia, and CF patients supplemented orally with vitamin E. It is evident that the majority of normal subjects ranged from 500 to 1,000 μg/dl plasma; this is in agreement with results noted in surveys of large populations, which have established 500 μg/dl as the lower limit of normal (20, 21). All CF patients with manifestations of pancreatic insufficiency showed plasma α-tocopherol concentrations below this level. Thus, there was no overlap in values between controls and patients with significant pancreatic involvement.

In addition to tocopherol determinations in plasma, we had an opportunity to evaluate necropsy samples of five tissues from one unsupplemented patient with steatorrhea. Analysis of liver, heart, skeletal muscle, lung, and fat revealed a markedly decreased α-tocopherol concentration in all samples (Table II). The degree of deficiency in these tissues was in agreement with the patient's very low plasma level (22 μg/dl).

Of particular interest were the two CF patients with intact or partially intact digestive function. A 25-yr-old female with 99% absorption of dietary fat, an unaltered serum carotene concentration (98 μg/dl), and normal pancreatic enzyme activities was found to have a plasma α-tocopherol concentration of 586 μg/dl. This value clearly falls in the normal range, an observation confirmed by determinations on two subsequent occasions. The second patient, a 26-yr-old male with borderline fat absorption (94%), a normal serum carotene level (74 μg/dl), and deficient trypsin, chymotrypsin, and carboxypeptidase B in duodenal fluid showed a plasma α-tocopherol concentration of 432 μg/dl. This represents the second highest value we observed in unsupplemented CF patients and is only about 10% below the lower limit of normal.

A graphical comparison between plasma α-tocopherol concentrations in unsupplemented CF patients and their degree of malabsorption is shown in Fig. 2. It was found that a significant correlation ($r = 0.96$) exists between circulating α-tocopherol and carotene. For patients with clinically significant steatorrhea, there was also a significant relationship ($r = 0.67$) between the percentage of dietary fat absorbed and the concentration of α-tocopherol in plasma.

Vitamin E status after oral supplementation with water-miscible tocopherol. The results of plasma α-tocopherol analyses in CF patients with steatorrhea who were given dietary supplements of vitamin E are shown in Fig. 1. In contrast to unsupplemented patients, those receiving a water-miscible form of vitamin E in dosages ranging from 50 to 400 IU/day were found in nearly all cases to have normal plasma

TABLE II

The α-Tocopherol Content of Organs Obtained at Autopsy from CF Patients

Subjects	Age	Tissue α-tocopherol, μg/g*				
		Liver	Heart	Muscle‡	Lung	Fat§
	yr					
Controls‖						
Mean±SE	44±3	33.5±12	20.9±3.0	19.5±3.7	11.4±2.1	298±82
Range	35–54	18.0–82.0	12.7–28.9	10.0–32.7	3.9–16.8	74.9–629
CF patients						
Case 1- not supplemented	25	ND¶	1.16	0.9	ND¶	ND¶
Case 2- supplemented**	29	20.2	—‡‡	9.50	—‡‡	—‡‡
Case 3- supplemented**	23	23.9	19.3	6.56	9.59	39.7
Case 4- supplemented**	19	29.0	19.1	13.0	—‡‡	189

* Measured according to the method of Bieri (12).

‡ Psoas muscle.

§ Pericardial or subcutaneous adipose tissue.

‖ Individual values have been reported previously by Bieri and Evarts (33); their data on six adults were used to calculate means and standard errors.

¶ ND, none detectable.

** Supplemented with 200 IU/day of α-tocopheryl acetate for 1 yr or more before death.

‡‡ Analysis was not performed.

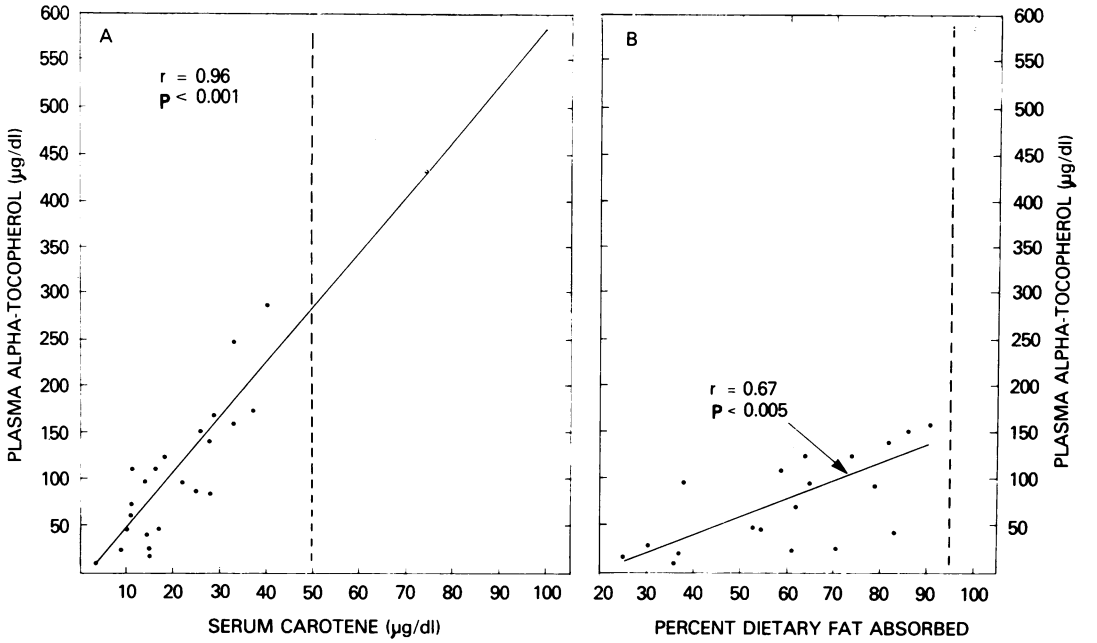

FIGURE 2 The correlation between plasma α-tocopherol concentrations in CF patients and their degree of malabsorption. The latter was evaluated by measurement of serum carotene concentration and fecal fat excretion during balance studies. The lower limits of normal for serum carotene (50 µg/dl) and for fat absorption (95%) are indicated by the broken lines. Open circles represent two CF patients with intact or partially intact digestive function, as assessed with these indices plus assay of duodenal contents.

α-tocopherol concentrations. The scatter diagram showing individual values, however, indicates that a wide range was found in the group of tocopherol-supplemented CF patients. Calculation of the mean concentration in 21 subjects who either were ingesting supplements of vitamin E when first evaluated or began treatment during this study revealed a value of 800±70 µg/dl plasma; this does not differ significantly from the control level of 683±33 µg/dl.

The results of tissue α-tocopherol determinations in three subjects supplemented with 200 IU of vitamin E per day are listed in Table II. It is evident that most of the values observed for liver, heart, skeletal muscle, lung, and adipose tissue are within the range found in age-matched, normal subjects.

Erythrocyte tocopherol concentration and fatty acid composition. CF patients with steatorrhea were found to show a mean α-tocopherol concentration in erythrocytes equal to 42 µg/dl of packed cells, which is significantly lower ($P < 0.001$) than the control group (Table III). Comparing erythrocytes with plasma in terms of the magnitude of diminished tocopherol revealed that CF patients manifest a less pronounced decrease in the cellular fraction of blood. Thus, erythrocyte vitamin E was 18% of the control mean, whereas the plasma level fell to 5% of the control value in these patients. This difference resulted in a significantly elevated erythrocyte:plasma tocopherol ratio in unsupplemented CF patients compared to controls (Table III).

Evaluation of erythrocyte fatty acid composition indicated that the major abnormalities in CF erythrocytes are a relatively high content of palmitoleate and oleate and a lower concentration of linoleate. In addition, erythrocytes from the vitamin E-deficient patients showed a higher ($P < 0.001$) proportion of homo-γ-linolenic acid (20:3ω6) and minor differences in other fatty acids, which were not statistically significant. Because of the reduction in linoleic acid and slightly lower amounts of other polyunsaturated fatty acids, the total polyunsaturated fatty acid content of CF erythrocytes was lower than the controls. There was no statistical difference, however, in the peroxidizable indices of the two groups (Table III), suggesting that the relative peroxidizability of erythrocyte fatty acids is not altered in CF.

Susceptibility of vitamin E-deficient erythrocytes to oxidation in vitro. Potential biological effects of vitamin E deficiency in erythrocytes were assessed in part by the peroxide hemolysis test in vitro. As mentioned previously, the procedure was modified from earlier techniques (14, 22) to improve its reliability and reproducibility. Preliminary studies, carried out to establish optimal conditions for this test indicated the following: (*a*) incubation time is an extremely important variable in that hemolysis at 37°C is not

TABLE III

*The α-Tocopherol Concentration and Fatty Acid Composition of Erythrocytes from CF Patients**

	Control‡	CF§
RBC α-tocopherol, *μg/dl cells*	239±15	42±17‖
Plasma α-tocopherol, *μg/dl*	1,055±104	50±20‖
RBC/Plasma tocopherol ratio	0.244±0.022	0.722±0.077‖
Fatty acids:		
Saturated	33.7±0.5	35.5±1.3
16+18 alds.	6.3±0.6	5.2±0.5
16:1ω7+18:1ω9	15.6±0.3	17.9±0.9¶
18:2ω6	9.9±0.2	7.1±0.3‖
18:3ω3	0.5±0.1	0.4±0.1
20:3ω6	1.3±0.1	2.1±0.1‖
20:4ω6	18.2±0.5	17.1±0.6
22:4ω6	4.6±0.2	4.3±0.5
22:5ω6	1.0±0.1	1.4±0.3
22:5ω3	2.8±0.3	2.2±0.2
22:6ω3	5.1±0.2	5.2±0.8
Total polyunsaturated fatty acid	43.5±0.8	39.8±0.9‖
Peroxidizable index	168±4	161±4

* Mean±SE values are shown.

‡ This group consisted of 11 healthy adults. Most of the individual values for these subjects were reported previously by Bieri and Poukka (13).

§ This group consisted of five CF patients with pancreatic insufficiency.

‖ $P < 0.001$ as compared to the corresponding value in controls.

¶ $P < 0.01$ as compared to the corresponding value in controls.

complete until 3 h elapse; and (*b*) maximum hemolysis is not achieved unless a H_2O_2 concentration of greater than 1% is utilized. To insure adequate peroxide availability, we chose a final concentration of 2% H_2O_2; this provides a slight excess but does not produce hemolysis in control erythrocyte (RBC) suspensions. Other modifications of previously described procedures which were of importance in assuring reproducible test results include: (*a*) rapid processing of erythrocyte samples; (*b*) use of disposable, plastic test tubes; (*c*) gentle, uniform mixing of suspensions by two inversions of parafilm-covered tubes; (*d*) no mixing during the 3-h incubation period; and (*e*) use of the same stock 30% solution of hydrogen peroxide for all hemolysis tests conducted during this phase of the study.

Once the optimal conditions were determined, the routine test procedure was utilized to evaluate blood samples from all CF patients visiting the National Institutes of Health Clinical Center during a 5-mo interval. This included 31 subjects with pancreatic insufficiency who were not receiving supplements of vitamin E; none of these patients were taking salicylates or were iron deficient, both of which can influence hemolysis test results. The degree of RBC hemolysis in these subjects ranged from 5 to 98% with a mean ±SE of 78±4.5%. This value is significantly higher ($P < 0.001$) than that of 32 controls (mean = 0.53 ±0.12; range = 0–2%). In contrast, the one CF patient with normal pancreatic function and all vitamin E-supplemented patients showed less than 2% hemoglobin release during 3-h incubations.

The relationship between the percentage of RBC hemolysis in H_2O_2 and the concentration of α-tocopherol in plasma is shown in Fig. 3. It is evident that significant hemolysis (>2%) occurred in all samples with less than 400 μg α-tocopherol per dl plasma. There is a relatively abrupt change from α-tocopherol levels which permit a low degree of hemolysis (300–400 μg/dl) to those at which hemolysis is extensive (<200 μg/dl). Thus, the relationship between the degree of hemolysis and the plasma α-tocopherol concentration is sigmoidal in nature.

Hemolysis in vivo measured after ^{51}Cr-labeling of erythrocytes. As shown in Table IV, 19 vitamin E-deficient CF patients were evaluated in terms of ^{51}Cr-RBC survival and compared to 28 control subjects who showed values ranging from 25 to 35 days. The vitamin E- deficient group was found to have a significantly decreased mean ^{51}Cr-RBC $t_{\frac{1}{2}}$ value of 22.4 days and a range of 15.5–29 days. Three CF patients were particularly low with values of 15.5, 16.0, and 18.0 days. Four subjects were in the normal range, however, and 52% of the values in the CF group were between 20 and 23 days, and thus only mildly shortened. No correlation could be found in comparing the extent of shortened ^{51}Cr-RBC survival with the degree of vitamin E deficiency.

In six patients, it was possible to assess ^{51}Cr-RBC survivals both in the vitamin E-deficient state and after supplementation with 200 IU/day of α-tocopheryl acetate. Before treatment, when the plasma α-tocopherol level averaged 80.7 μg/dl and hemolysis in vitro

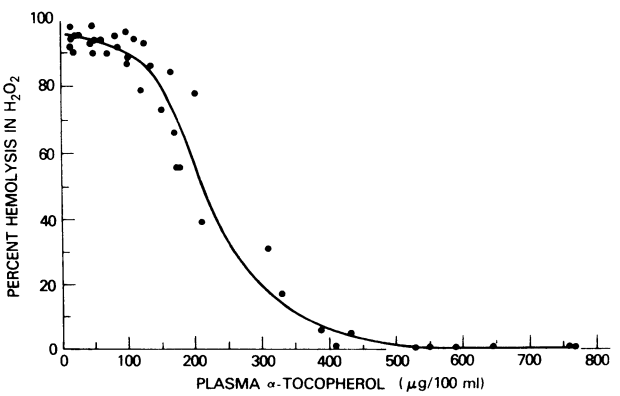

FIGURE 3 The relationship between the degree of peroxide-induced hemolysis and the plasma α-tocopherol level in CF patients. The sigmoid curve was drawn to approximate the apparent trend of the data.

84%, the group showed a ^{51}Cr-$t_{\frac{1}{2}}$ value of 19.0±1.3 days (mean±SE). This was significantly increased to 27.5±0.9 days after supplementation, when all of the subjects showed lower hemolysis in vitro as well, and a corresponding rise in plasma α-tocopherol concentration.

Other hematologic indices before and after supplementation with vitamin E. A group of 14 CF patients with low plasma tocopherol concentrations (mean = 84±12 μg/dl) who were in a relatively stable condition were evaluated for 2 yr in regard to hematologic status. During the 1st yr, while in the vitamin E-deficient state, hemoglobin and hematocrit values, RBC counts, and reticulocyte counts were measured on two or three occasions. None of the patients was found to have either anemia or presistent reticulocytosis (mean hemoglobin = 14.3 μg/dl). Each member of this group then consumed 100–200 IU/day of vitamin E for 1 yr, during which time all patients showed a rise in plasma α-tocopherol to normal levels (mean ±SE = 780±68; $P < 0.001$, as compared to the pretreatment level). There was no change, however, in any of the hematologic indices.

DISCUSSION

Studies of human vitamin E deficiency are limited to two populations in developed countries, premature infants and patients with intestinal malabsorption. A number of malabsorptive states have attracted interest relative to vitamin E. These include CF, hepatobiliary disturbances such as biliary atresia, celiac disease, intestinal lymphangectasia, and a-betalipoproteinemia (1, 23). Relatively few patients with each of these disorders, however, have been characterized in terms of vitamin E status, and those who have been studied exhibit an unexplained wide range of blood tocopherol levels. Assessment of vitamin E status in such patients has generally been carried out with methods that measure total tocopherols (α-, β-, and γ-isomers) in plasma or serum (1, 7–9). Although satisfactory for screening purposes, they overestimate the true circulating vitamin E level by 10–20%; and in patients taking drugs, the error may be greater. In addition, none of the previous investigations have delineated tocopherol:lipid ratios (10), and few have documented the extent of fat malabsorption in individual subjects.

Because of their chronic steatorrhea, patients with CF are particularly suitable for evaluation of the degree and effects of vitamin E deficiency in man. Focusing on this malabsorption syndrome, the present study demonstrates that CF patients with pancreatic achylia are uniformly low in plasma α-tocopherol, unless they are ingesting large dietary supplements of the vitamin. The observation that these patients are truly deficient in vitamin E was confirmed by measuring

TABLE IV
Erythrocyte Survival in Control Subjects and Vitamin E-Deficient CF Patients

Group	Number	^{51}Cr-RBC half-life, *days*	
		Mean±SE	Range
	n		
Control	28	28.0±0.5	25.0–35.0
Vitamin E deficient*	19	22.4±0.9‡	15.5–29.0

* Plasma α-tocopherol concentration in this group was 86.2±15 μg/dl.
‡ $P < 0.001$ as compared to the control subjects.

tocopherol:lipid ratios in plasma, the α-tocopherol content of erythrocytes and various tissues, and the hemolysis of CF erythrocytes in hydrogen peroxide. Underwood and associates (24, 25) previously noted diminished tocopherol levels in erythrocytes, liver, and muscle from CF patients. Our finding of low concentrations in lung and adipose tissue, however, represents a new observation, which is of interest and possible importance in view of the severe pulmonary disease accompanying CF. Although the degree of vitamin E deficiency, as indicated by the magnitude of diminished plasma α-tocopherol, was found to vary somewhat in our population of subjects with malabsorption, the majority displayed levels below 100 μg/dl plasma. We therefore conclude that most unsupplemented CF patients are markedly deficient, such that their erythrocytes are almost completely unprotected from the oxidative stress of exposure to peroxide.

The wide range of blood vitamin E concentrations observed in previous studies of CF patients (1, 8), as well as in this investigation, prompted us to compared the severity of impaired digestion to the degree of vitamin E deficiency. Results of comparing two indices of malabsorption, i.e., fecal fat excretion and the serum carotene concentration, with plasma α-tocopherol levels in individual patients revealed a good correlation for each. Accordingly, it is proposed that vitamin E deficiency in CF patients occurs to an extent determined by the degree of malabsorption. Further investigation of tocopherol malabsorption in CF would require the use of radioactive vitamin E preparations. This direct method was utilized by MacMahon and Neale (26), who conducted a study administering tritiated α-tocopherol orally to patients with malabsorptive disorders other than CF. Noteworthy is their observation that the degree of tocopherol malabsorption could be correlated statistically with the magnitude of fecal fat excretion. This supports the proposal that impaired absorption of tocopherol occurs to an extent determined by the degree of steatorrhea, an hypothesis in keeping with the current belief that intestinal uptake of vitamin E is

dependent upon the ability to digest and absorb dietary triglyceride in general (27).

A group of CF patients consuming supplements of vitamin E when this investigation was initiated, and the willingness of several additional subjects to cooperate for a trial of vitamin E administration, permitted us to assess the adequacy of dietary supplementation. It was found that ingestion of an appropriate preparation of α-tocopherol in doses above the recommended daily allowance overcomes the deficiency state in nearly all cases. Indeed, supplements of 100–200 IU/day were successful in raising plasma α-tocopherol to concentrations indistinguishable from those found in controls. Furthermore, by measuring tissue tocopherol concentrations in supplemented patients, it was shown for the first time that these levels can also be raised to normal in CF patients.

Regarding the effects of vitamin E deficiency, this investigation focused on possible hematologic abnormalities. Previous studies have identified abnormal erythrocyte hemolysis in vitro in many, but not all, of the CF patients examined with low plasma tocopherol concentrations (1, 22). In addition, earlier reports have noted that peroxide hemolysis test results are difficult to reproduce and that samples from healthy subjects with normal tocopherol levels may show as much as 10–20% hemolysis (1, 14, 22). Because of the difficulties encountered with other hemolysis test procedures, we devoted considerable effort in this study to establishing conditions in which: (*a*) reproducible results could be obtained with tocopherol-deficient erythrocytes, and (*b*) control preparations would not hemolyze. With the modifications described in the present report, such a test was developed and found to be useful for our purposes. In particular, it was possible in this study to determine the precise relationship between percentage hemolysis and the plasma α-tocopherol concentration, a relationship which could not be established, beyond the finding of an inverse correlation, in earlier examinations of human erythrocytes (conducted primarily with blood from premature infants) (28). Our results indicate that when the data are plotted in a rectilinear fashion (Fig. 3), the hemolysis-tocopherol curve is best approximated by a sigmoidal function. Such a relationship was observed previously with rat erythrocytes (19, 29) and suggests that a threshold concentration of vitamin E exists in the erythrocyte below which antioxidant protective activity is virtually exhausted.

There has been considerable disagreement on the question of whether or not significant shortening of the erythrocyte life-span occurs in human tocopherol deficiency (1, 30–32). The relatively large population of clinically stable CF patients followed at the National Institutes of Health afforded us an opportunity to examine the issue of in vivo hemolysis in greater detail than was possible previously. Erythrocyte survival determinations in vitamin E-deficient subjects revealed that their erythrocytes were abnormal with a mean $t_{\frac{1}{2}}$ value of 22.4 days. It should be recognized that this degree of decreased erythrocyte life-span, although statistically significant, is not sufficient to produce frank hemolytic anemia, where values of 5–15 days are frequently observed. This was substantiated by careful examination of hematologic indices which failed to disclose unexplained anemia in any of our 52 CF patients with vitamin E deficiency. Of greatest interest was the finding that repeat ^{51}Cr-RBC survival measurements after supplementation with α-tocopherol disclosed a significant increase in $t_{\frac{1}{2}}$ from 19.0 days to 27.5 days after treatment. This observation provides good evidence that patients with steatorrhea and tocopherol deficiency require vitamin E for maintenance of normal erythrocyte function.

Viewed collectively, observations from our study and from others dealing with vitamin E-deficient patients support the following conclusions: (*a*) CF patients with complete pancreatic insufficiency are uniformly, and in most cases profoundly, deficient in plasma α-tocopherol. (*b*) The degree of vitamin E deficiency in patients with long-term steatorrhea occurs to an extent determined by the severity of malabsorption. (*c*) CF in the absence of significant pancreatic involvement is not accompanied by vitamin E deficiency. (*d*) Erythrocytes from CF patients with pancreatic achylia are low in α-tocopherol, but the reduction does not occur to as great an extent as in plasma. Thus, the RBC:plasma tocopherol ratio is higher in deficient CF patients compared to controls. (*e*) Supplements of water-miscible α-tocopheryl acetate will overcome vitamin E deficiency in CF patients and produce normal tocopherol concentrations in both plasma and tissues. (*f*) With improvements in the peroxide hemolysis test procedure, a sigmoidal relationship between susceptibility of human erythrocytes to H_2O_2 and the concentration of tocopherol in plasma was demonstrated for the first time. All CF patients with low blood tocopherol levels show an abnormal degree of hemolysis in vitro when erythrocytes are exposed to oxidant stress. (*g*) In vivo, however, ^{51}Cr-RBC survival is only mildly to moderately shortened in vitamin E-deficient patients, and not to a degree where hemolytic anemia would occur. The effect of vitamin E deficiency on the hematologic system is, therefore, a subclinical one. Nevertheless, the demonstration of a statistically significant decrease in ^{51}Cr-RBC half-life, and especially the finding that erythrocyte survival can be corrected upon tocopherol therapy, provides strong evidence that such patients, hence humans in general, require vitamin E for maintenance of normal erythrocyte function.

The metabolic role of this ubiquitous biological antioxidant is still unclear, but its widespread presence

in human tissues indicates that it must serve an essential purpose. Even though clinical symptoms of vitamin E deficiency are not evident in patients with CF and other conditions accompanied by steatorrhea, it is reasonable to speculate that diminished antioxidant levels and a shortened erythrocyte life-span place excessive depands on cellular metabolism. Patients with malabsorption and secondary tocopherol deficiency should therefore be given regular supplements of vitamin E.

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