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The relative importance of the factors involved in the absorption of vitamin E in children

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SUMMARY The vitamin E status and ease of repletion in groups of children with coeliac disease, intestinal lymphangiectasia, and abetalipoproteinaemia was studied and compared with earlier studies in cystic fibrosis and obstructive jaundice. Each group represents an experimental model in which one of the transport steps involved in the absorption of vitamin E is defective or absent and thus the relative importance of these factors could be determined. Chylomicron formation and an adequate intraluminal concentration of bile salts were found to be the most important factors for the efficient absorption of the vitamin. The results in the five groups of patients have therapeutic implications if it is considered desirable to correct vitamin E deficiency states.

Reduced serum concentrations of vitamin E have been reported in adults with a variety of malabsorption syndromes (Binder, Herting, Hurst, Finch, and Spiro, 1965; Losowsky and Leonard, 1967; Kelleher and Losowsky, 1970). In children similar but less extensive observations have been made; low levels have been described in cystic fibrosis (Bennett and Medwadowski, 1967) and other malabsorptive states (Nitowsky, Cornblath, and Gordon, 1956). In abetalipoproteinaemia the vitamin is undetectable in the serum (Ways and Simon, 1964; Kayden, Silber, and Kossman, 1965; Dodge, Cohen, Kayden, and Phillips, 1967). Despite these observations, however, knowledge of the relative importance of the factors involved in the absorption of vitamin E is limited.

We have already shown that children with pancreatic insufficiency (Harries and Muller, 1971a) or biliary obstruction (Harries and Muller, 1971b) have malabsorption of vitamin E and in the present study we compare the degree of vitamin E deficiency in these children with that found in children with malabsorption resulting from coeliac disease, intestinal lymphangiectasia, or abetalipoproteinaemia.

The results provide a basis for comparing the relative importance of the factors involved in the absorption of vitamin E, a knowledge of which has important therapeutic implications for the correction of vitamin E deficiency states.

Patients and Methods

PATIENTS

The number of children and their ages when first studied are given in table I.

Diagnosis	Mechanism for Vitamin E Malabsorption	No.	Age Range (yr)
Coeliac disease	Abnormalities of proximal absorptive cells	13	0.7-14
Intestinal lymphangiectasia	Impaired lymph flow	4	1.5-10
Abetalipoproteinaemia	Failure to form chylomicra	8	0.1-10

Table I *Patients studied*

The diagnosis of coeliac disease was established by peroral jejunal biopsy and clinical response to a gluten-free diet.

All the children with intestinal lymphangiectasia had evidence of protein-losing enteropathy as assessed by intestinal loss of ⁵¹Cr-labelled albumin; the diagnosis was established by intestinal biopsy in three cases and in the other two the clinical, radiological, and laboratory findings were characteristic. Treatment consisted of a diet low in long-chain fatty acids (< 10 g total fat per day) supplemented with medium-chain triglyceride.

The children with abetalipoproteinaemia presented with malabsorption of fat, acanthocytosis of the red cells, and low total serum cholesterol concentrations (19.45 mg/100 ml). The diagnosis was confirmed by the failure to detect betalipoprotein in the serum by electrophoretic, ultracentrifugal, and immunochemical studies. All the children were treated with low-fat diets (1.20 g/day) and two received additional medium chain triglyceride supplements.

METHODS

The vitamin E status was assessed by estimating serum concentrations of vitamin E and by two tests of red cell haemolysis, autohaemolysis, and peroxide haemolysis. Blood was obtained by venepuncture and on most occasions vitamin E and haemolysis were estimated on the same sample. Tests for haemolysis were commenced immediately. Serum for vitamin E estimations was stored at -20°C for a maximum of four weeks. During this time there is no change in serum concentrations (Muller, 1971).

Serum vitamin E was measured in triplicate on aliquots of 0.3 ml serum essentially as described by Quaife, Scrimshaw, and Lowry (1949) but with the following modifications; bathophenanthroline was used as the indicator (Tsen, 1961), the amount of beta-carotene (the principal interfering substance) was estimated at 450 nm, and the appropriate correction made (Bieri, Teets, Belavady, and Andrews, 1964), and orthophosphoric acid was added to minimize the photoreduction of ferric ions (Tsen, 1961).

Autohaemolysis was measured by the method of Young, Izzo, Altman, and Swisher (1956) and peroxide haemolysis by the procedure of Gordon, Nitowsky, and Cornblath (1955). In the earlier studies the former method was used; subsequently peroxide haemolysis was preferred as it was more sensitive, required less blood (0.2 ml compared with 6.0 ml), and was quicker to perform (five hours compared with two days).

The mean concentration of vitamin E ± 1 SD in 21 children who were of good nutritional status and with no evidence of malabsorption was 0.80 mg/100 ml ± 0.18 , and their mean percentage peroxide haemolysis 2 ± 2.2 .

ADMINISTRATION OF VITAMIN E

The vitamin was given as DL-alpha tocopheryl acetate either intramuscularly¹ or orally. Two oral preparations were used: (a) a fat-soluble preparation in tablet form (Ephynal¹), or (b) a water-miscible preparation.¹

¹Roche Products Limited.

Results

COELIAC DISEASE

Vitamin E status was assessed before the introduction of a gluten-free diet in 13 children. The mean serum vitamin E concentration was 0.39 mg/100 ml ± 0.20 and the mean percentage peroxide haemolysis in nine of them was 13 ± 12 (fig 1).

In four children serum vitamin E concentrations were estimated before and after the introduction of a gluten-free diet. None of the children received any vitamin E supplements at any stage of their treatment. The results are shown in table II. In all cases serum levels of vitamin E increased during the study period of five to 12 weeks, and in three of the children levels had returned to normal by this time.

INTESTINAL LYMPHANGIECTASIA

Before the administration of oral vitamin E supple-

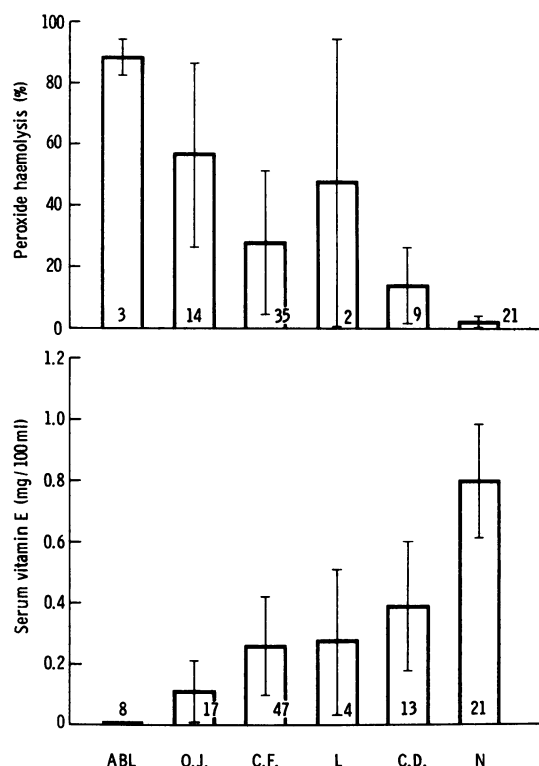


Fig 1 Mean percentage peroxide haemolysis and serum vitamin E concentrations (± 1 SD) in five groups of patients and a control group.

ABL—abetalipoproteinaemia; O.J.—obstructive jaundice; C.F.—cystic fibrosis; L—intestinal lymphangiectasia; C.D.—coeliac disease; and N—normal controls.

Patient	Serum Vitamin E (mg/100 ml)		Time on Diet (wk)
	Before	After	
1	0.22	0.83	8
2	0.32	0.42	12
3	0.29	0.93	8
4	0.13	0.50	5

Table II Serum vitamin concentrations in children with coeliac disease before and after introduction of a gluten-free diet

ments the mean serum concentration in these four children was $0.28 \text{ mg/100 ml} \pm 0.24$ (fig 1). Levels in two of the children were documented before the introduction of a low-fat diet and their serum concentrations were 0.04 and 0.14 mg/100 ml.

Figure 2 shows the response to long-term oral administration of the fat-soluble preparation of vitamin E in one child. The vitamin was given in doses of 0.5, 10, and 15 mg/kg/day for varying periods of time. The abnormal haemolysis was corrected during each of the three treatment periods and again became abnormal after discontinuing therapy. Serum vitamin E increased during the three treatment periods, but normal levels were only achieved with the larger doses.

ABETALIPOPROTEINAEMIA

In all eight children serum levels of vitamin E were undetectable before administration of the vitamin (fig 1). Autohaemolysis was increased in four

patients and peroxide haemolysis in three (studies were not possible in one child).

One child was given an oral load of the water-miscible preparation of vitamin E (120 mg/kg) together with a meal containing fat, but no increase in the serum vitamin E concentration was detected over the subsequent 24 hours. Acute intramuscular loads (45 mg/kg over 48 hours and 12.5 mg/kg in a single dose) were given to two children; both responded similarly and the response of the latter is illustrated in fig 3 where it is compared with a comparable intramuscular dose in a child with obstructive jaundice. Peroxide haemolysis returned to normal within 24 hours in both children. Serum vitamin E increased to similar levels (0.35 mg/100 ml) by five to six hours, but no further rise was observed in the child with abetalipoproteinaemia, whereas levels in the child with obstructive jaundice continued to rise to well within the normal range.

Because it was not considered justifiable to treat children with abetalipoproteinaemia with long-term intramuscular vitamin E, they were all given oral supplements in large doses of approximately 100 mg/kg/day. Initially the water-miscible preparation was used; later, however, it became clear that the fat-soluble preparation was equally effective (fig 4) and subsequently this preparation was used except for children who were unable to take the large number of tablets required. For these the water-miscible preparation was more convenient. Serum vitamin E concentrations and percentage haemolysis fluctuated over the years during which regular observations were made; only the maximum vitamin

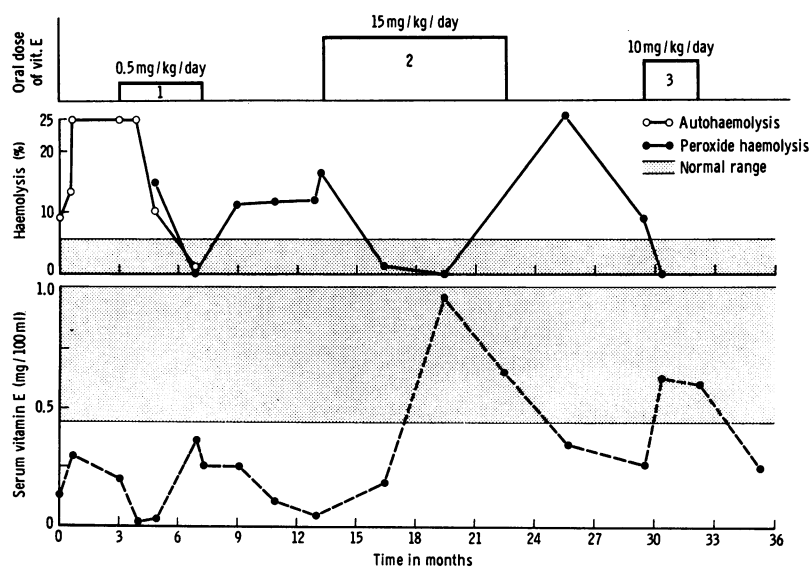


Fig 2 Response of a child with intestinal lymphangiectasia to three different oral doses of a fat-soluble preparation of vitamin E.

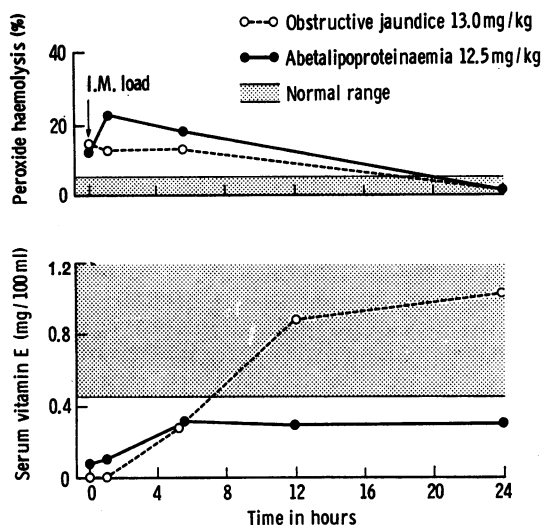


Fig 3 Responses to comparable single intramuscular injections of vitamin E in abetalipoproteinaemia and obstructive jaundice.

E and minimum haemolysis values obtained are given in table III. In all eight children the vitamin could be detected in the serum within one year of starting treatment and in five the maximum levels were similar to those obtained after intramuscular injection. The percentage haemolysis fell during vitamin E therapy in all the children in whom it was estimated, and in seven of the eight children the minimum levels achieved during vitamin E supplementation were within the normal range.

Discussion

A comparison of the degree of deficiency and ease of repletion of vitamin E in the three groups of children described in this paper (coeliac disease, intestinal

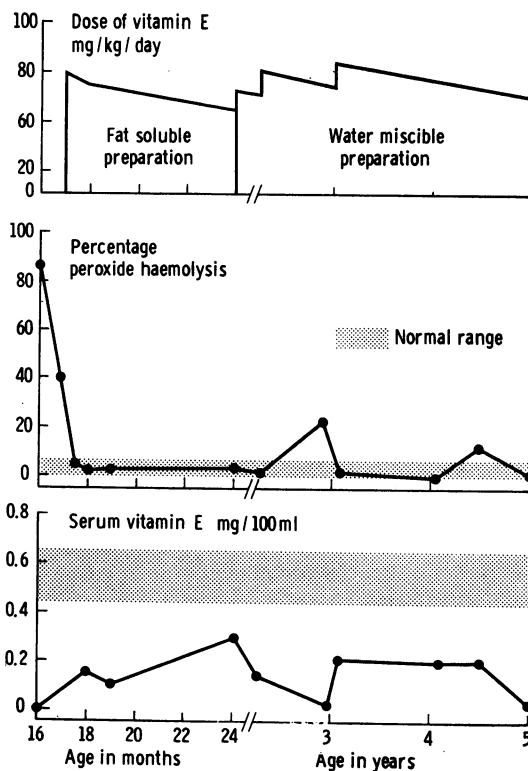


Fig 4 Response to fat-soluble and water-miscible preparations of vitamin E in abetalipoproteinaemia.

lymphangiectasia, and abetalipoproteinaemia), combined with the results of our earlier studies in cystic fibrosis and obstructive jaundice (Harries and Muller, 1971a and b), provide information on the relative importance of the factors involved in the absorption of this vitamin. Each condition can be taken to represent an experimental model in which one of the intestinal transport steps involved in the

Patient	Age at Start of Vitamin E Therapy		Duration of Vitamin E Therapy to Date		Serum Vitamin E (mg/100 ml)		Percentage Peroxide Haemolysis	
	Years	Months	Years	Months	Before Treatment	Maximum after Treatment	Before Treatment	Maximum after Treatment
1		3	7	1	Nil	0.30	15 ¹	Nil
2		2	4	1	Nil	0.22	93	Nil
3	10	3	8	0	Nil	0.09	33 ¹	10
4		5	4	6	Nil	0.27	Not estimated	5
5		11	2	5	Nil	< 0.10	58	Nil
6	1	4	4	5	Nil	0.30	63	Nil
7	8	9	7	8	Nil	0.24	17 ¹	Nil
8	3	5	7	2	Nil	0.11	15 ¹	3
Normal range					0.44-1.5 mg/100 ml		< 6% (¹ autohaemolysis < 5%)	

Table III Long-term effect of oral vitamin E (approximately 100 mg/kg/day) in abetalipoproteinaemia

absorption of vitamin E is defective or absent. Deficiency was greatest in abetalipoproteinaemia and obstructive jaundice, suggesting that normal chylomicron formation and adequate intraluminal bile salt concentrations are of particular importance. Pancreatic enzymes, proximal mucosal integrity, and intestinal lymphatic flow appear to be relatively less important.

Abetalipoproteinaemia presents a unique model for studying the role of chylomicra on intestinal transport of vitamin E. The finding that before therapy the vitamin was undetectable in the serum and that haemolysis was raised in all the children in whom it was estimated is in agreement with previous studies (Ways and Simon, 1964; Kayden *et al*, 1965; Dodge *et al*, 1967), and suggests that chylomicron synthesis is very important for absorption of vitamin E. This is confirmed by the failure to detect vitamin E in the serum over a 24-hour period following a large oral load (120 mg/kg) given with fat. However, the correction of abnormal haemolysis and the increase in serum levels following intramuscular injections of vitamin E in two children show that vitamin E can be transported in the blood even in the absence of betalipoprotein, its principal carrier (McCormick, Cornwell, and Brown, 1960; Pelkonen, 1963; Kayden *et al*, 1965).

Contrary to the experience of other workers (Kayden *et al*, 1965; Dodge *et al*, 1967), we were able to demonstrate both correction of the abnormal peroxide haemolysis and the appearance of vitamin E in the serum of our patients following massive oral doses of the vitamin. Dodge *et al* had been able to correct the abnormal haemolysis but could not influence serum vitamin levels whereas Kayden *et al* had been unable to detect any change in either parameter. In neither of these studies, however, was any indication given of the dosage administered on a body weight basis, but from the total dose and age of the patients it was undoubtedly far less than the 100 mg/kg/day which we found to be necessary to achieve detectable serum concentrations.

Kayden *et al* (1965) and MacMahon, Neale, and Thompson (1971) have shown in the rat that the lymphatic pathway is the major route for transport of vitamin E out of the mucosal cell. The latter group, however, have also shown that a small proportion of vitamin E can be transported via the portal vein (MacMahon *et al*, 1971). In abetalipoproteinaemia, therefore, the small amounts of the vitamin which are absorbed are presumably transported by this route.

We have previously reported that serum levels of vitamin E are very low and repletion extremely difficult in patients with biliary obstruction (Harries

and Muller, 1971b). In six children with persistent obstruction no evidence of absorption could be detected even after the administration of very large doses (up to 270 mg/kg/day) of either the fat-soluble or water-miscible preparations of vitamin E for up to three years. The rapid return of serum levels and haemolysis to normal following intramuscular loads in three children provides evidence that the deficiency resulted from intestinal malabsorption. That impaired bile flow was the cause of this malabsorption was shown by the marked improvement which occurred in the patients who were studied when biliary obstruction was improving. Other workers have also shown that bile plays a major role in vitamin E absorption in man (Blomstrand and Forsgren, 1968; MacMahon and Neale, 1970) and in the experimental animal (Gallo-Torres, 1970).

The precise mechanisms by which reduced intraluminal concentrations of bile result in such severe malabsorption of vitamin E are not clear. Bile salts are important for intraluminal solubilization and absorption of dietary lipids (Hofmann and Borgström, 1962), and it is likely that such non-polar substances as the fat-soluble vitamins are particularly dependent on this system (Dawson, 1967; Badley, Murphy, Bouchier, and Sherlock, 1970). However, impaired intraluminal solubilization of vitamin E is unlikely to be the only explanation since, if this were the case, better absorption of the water-miscible preparation might have been expected. Gallo-Torres (1970) has shown in the rat that both pancreatic enzymes and intraluminal bile are essential for the hydrolysis and absorption of vitamin E esters and we have shown recently (Muller, unpublished) that bile salts are essential cofactors for the principal hydrolytic enzyme (carboxylic ester hydrolase). Thus bile salts appear to play a specific role in the hydrolysis of vitamin E esters which is an obligatory first step in the absorption of the vitamin.

Coeliac disease is a convenient model for studying small intestinal absorption sites since the mucosal abnormalities involve predominantly the proximal small intestine, the distal part usually being unaffected (Rubin, Brandborg, Phelps, and Taylor, 1960; Booth, 1970). In three of the four patients whom we were able to study sequentially serum vitamin E returned to normal within a few weeks of starting a gluten-free diet without additional vitamin E supplements. These observations suggest that dietary vitamin E was absorbed by the recovering mucosa, and that the proximal region of the small intestine is a relatively important site for vitamin E absorption.

Since vitamin E is normally absorbed via lymphatic channels (Kayden *et al*, 1965; MacMahon *et al*, 1971), impaired lymph flow might

be anticipated to result in malabsorption of the vitamin and this is presumably the explanation for the reduced serum vitamin E levels in the patients with intestinal lymphangiectasia. The reduced serum concentrations did not appear to result from treatment with a low-fat diet as the lowest concentrations were documented in two children before they were placed on the diet. In the one child studied over a prolonged period, the correction of abnormal red cell haemolysis and the rise in serum concentrations which followed the administration of small doses of vitamin E confirm that malabsorption in this condition is much less severe than in obstructive jaundice or abetalipoproteinaemia.

The vitamin E deficiency which occurs in cystic fibrosis (Bennett and Medwadowski, 1967; Harries and Muller, 1971a) probably results from a reduced mixed micellar phase and impaired solubilization of non-polar lipids such as the fat-soluble vitamins. Our earlier work (Harries and Muller, 1971a), which shows that a much smaller dose of the water-miscible compared with the fat-soluble preparation was required to achieve and maintain normal serum vitamin E concentrations, confirms this hypothesis.

An understanding of the absorption of vitamin E esters is clearly relevant to the treatment of deficiency states of this vitamin but apart from abetalipoproteinaemia (Lloyd and Muller, 1972) there is no evidence that the correction of low serum levels of this vitamin is of clinical benefit to children with malabsorption.

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