Absorption of vitamin E in children with biliary obstruction

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SUMMARY Serum vitamin E levels and red cell haemolysis were measured in 17 children with biliary obstruction after oral and intramuscular loading tests, and during long-term oral administration of differing doses of a fat-soluble and water-miscible preparation of alpha-tocopheryl acetate. The results suggested a severe defect in the intestinal absorption of both preparations. In three of the children who were studied during periods of improving biliary obstruction, absorption was shown to have improved. Bile plays a major role in the absorption of vitamin E from the intestinal tract and the exact mechanism of its action requires further elucidation.

In adults vitamin E deficiency can be associated with various types of liver disease (Klatskin and Molander, 1952; Klatskin, 1954; Binder, Herting, Hurst, Finch, and Spiro, 1965; Kater, Unterecker, Kim, and Davidson, 1970) but there has been disagreement regarding the relative importance of the mechanisms causing the deficiency. Impaired blood transport (Kater *et al.*, 1970), intestinal malabsorption (Popper, Dubin, Steigmann, and Hesser, 1949; Blomstrand and Forsgren, 1968; MacMahon and Neale, 1970), increased destruction within the intestinal lumen (Klatskin, 1954), and deficient dietary intake (Klatskin and Molander, 1952) have all been implicated as possible causes. Bile is known to be important for the efficient absorption of the fat-soluble vitamins as a group (Dawson, 1967) but only more recently has information become available on its specific influence on vitamin E absorption. The recent studies of Blomstrand and Forsgren (1968) and MacMahon and Neale (1970) suggest that bile plays a major role in the absorption of vitamin E in the adult and similar findings have been reported for the experimental animal (Gallo-Torres, 1970). In considering the effect of liver disease on vitamin E metabolism, particularly as regards its absorption, it would therefore seem important to establish the degree of biliary obstruction. The majority of patients studied by Popper *et al.* (1949), Klatskin and Molander (1952), and Kater *et al.* (1970) had cirrhosis, and recent evidence suggests that in the absence of steatorrhoea the output and concentration of bile salts are normal in this condition (Badley, Murphy, Bouchier, and Sherlock, 1970;

Turnberg and Graham, 1970). Except for the reports of Nitowsky and his colleagues (Nitowsky, Cornblath, and Gordon, 1956a; Nitowsky, Gordon, and Tildon, 1956b; Nitowsky, Tildon, Levin, and Gordon, 1962), which included two children with obstructive jaundice, we are not aware of other studies on vitamin E absorption in children with liver disease. This paper presents the results of a study designed to investigate the intestinal absorption of vitamin E in 17 children who had liver disease in association with a severe degree of biliary obstruction.

Patients and Methods

PATIENTS

The ages of the 17 patients at the time when their vitamin E status was first assessed ranged from 2 months to 15 years (mean 31 months) and only one had received vitamin E (15 mg orally/day for three weeks) before this time. The diagnosis of obstructive jaundice was based on clinical and biochemical evidence of liver disease: the main fraction of the raised serum bilirubin being conjugated, the absence of detectable bile pigment in the stools, and the presence of excess bile pigment in the urine. The causes of the obstructive jaundice are shown in Table I. In all cases the diagnosis was based on exploratory laparotomy together with operative cholangiograms and liver biopsies in most instances. In 13 of the children biliary obstruction persisted throughout the period of study, whereas in the other four patients biliary obstruction improved. Dietary intake of fat was reduced in patients S.J., S.G., D.B.,

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Patient	Cause of Obstructive Jaundice
M.H.	Extrahepatic biliary atresia
A.H.	Extrahepatic biliary atresia
K.L.	Extra- and intrahepatic biliary atresia
S.J.	Extrahepatic biliary atresia; cirrhosis
D.B.	Neonatal hepatitis of undetermined cause; cirrhosis
S.K.	Extrahepatic biliary atresia
L.H.	Cirrhosis; ?extrahepatic biliary atresia
M.T.	Intrahepatic biliary atresia
H.P.	Intrahepatic biliary atresia; cirrhosis
P.N.	Partial intrahepatic biliary atresia; cirrhosis
G.C.	Neonatal hepatitis of undetermined cause
S.G.	Neonatal hepatitis; transient tyrosinosis
M.L.	Neonatal hepatitis due to CMV virus; cirrhosis
J.G.	Neonatal hepatitis of undetermined cause
J.K.	Cirrhosis
J.H.	Cirrhosis
P.H.	Extreme prematurity

Table I Cause of obstructive jaundice

M.L., P.N., M.T., and K.L.; in addition the diets of K.L., J.H., and M.T. contained medium-chain triglycerides.

METHODS

The vitamin E status of the children was assessed by estimating serum concentrations of vitamin E and by two tests of red cell haemolysis, autohaemolysis, and peroxide haemolysis. Serum vitamin E was determined in triplicate on aliquots of 0.3 ml of serum by the method of Quaife, Scrimshaw, and Lowry (1949) with the following modifications: bathophenanthroline (Tsen, 1961) was substituted for the indicator alpha, alpha dipiridyl reagent; the principal interfering substance, beta carotene, was estimated at 450 m μ and the appropriate correction made (Bieri, Briggs, Pollard, and Fox, 1960) and orthophosphoric acid was added to minimize the photoreduction of ferric ions (Tsen, 1961). In addition, the solution was protected from direct sunlight by a covering of aluminium foil which resulted in better agreement between all the triplicates and the reagent blanks. Blood was obtained by venepuncture after an overnight fast, and on most occasions both serum vitamin E and peroxide haemolysis were measured on the same sample. Serum was stored at -20°C and vitamin E estimated within four weeks of collection since previous experiments had shown that no deterioration of alpha tocopherol could be detected in serum stored for this length of time.

Autohaemolysis was measured by the method of Young, Izzo, Altman, and Swisher (1956). Peroxide haemolysis was estimated by the method of Gordon, Nitowsky, and Cornblath (1955). Increased haemolysis (peroxide haemolysis > 6%; autohaemolysis > 5%) by either test could be corrected by the addition of normal serum, or physiological quantities of alpha-tocopherol to the *in-vitro* system. In the initial

studies autohaemolysis was measured, but it was later found that peroxide haemolysis was a far more sensitive index of vitamin E deficiency and had the added advantage of requiring much smaller volumes (0.2 ml) of blood compared with autohaemolysis (5-6 ml). This method was, therefore, adopted for routine use.

Vitamin E was administered as DL-alpha-tocopheryl acetate¹ whether it was given by the oral or intramuscular route. Two oral preparations were used: a fat-soluble preparation in tablet form (Ephynal) and a water-miscible preparation containing a surface active agent (Cremaphor EL, at a concentration of 16 g/100 ml), and glycerine.

Absorption was investigated in three ways: (1) oral load tests, (2) intramuscular load tests, and (3) long-term oral administration.

1 Oral load tests

The oral loads of vitamin E were given after an overnight fast and the usual dietary regime was maintained during the test period. D.B. received the water-miscible preparation in a dose of 26 mg/kg, and H.P. was given both the water-miscible (38 mg/kg) and fat-soluble preparations (30 mg/kg) with an interval of four days between each load. Serum vitamin E was estimated immediately before and three, six, and 12 hours after the load. In addition, peroxide haemolysis was measured in D.B.

2 Intramuscular load tests

M.T., M.L., and P.H. each received a single intramuscular injection of alpha-tocopheryl acetate in doses of 13, 24, and 40 mg/kg body weight respectively. Serum vitamin E and peroxide haemolysis were followed for periods of 12, 17, and 24 days after the loading dose.

3 Long-term oral administration of vitamin E

Longitudinal studies were performed in 10 of the 17 children. The children fell into one of two clinical groups: six patients in whom biliary obstruction persisted throughout the period of study, and four patients in whom biliary obstruction improved during the period of study.

Results

Figure 1 shows the correlation between serum vitamin E levels and peroxide haemolysis in 14 children with obstructive jaundice compared with that in 22 'control' children who did not have malabsorption or other evidence of nutritional disease. In all children both tests were done on the same sample of blood and none had received

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Fig. 1 Relationship between peroxide haemolysis and serum concentrations of vitamin E in 64 children with biliary obstruction compared with 21 children of good nutritional status without malabsorption.

vitamin E. All the patients with obstructive jaundice had low serum levels of vitamin E (means $0.11 \text{ mg}/100 \text{ ml} \pm 0.11 \text{ ISD}$) and increased peroxide haemolysis (mean $57.1\% \pm 28.1$) compared with the children without malabsorption (mean serum vitamin E $0.80 \text{ mg}/100 \text{ ml} \pm 0.18$, and mean peroxide haemolysis $1.9\% \pm 2.2$).

1 ORAL LOAD TESTS

Despite the large doses of vitamin E given, no rise occurred in serum levels of vitamin E in any of the three children, and in D.B. peroxide haemolysis also remained grossly abnormal.

2 INTRAMUSCULAR LOAD TESTS

In contrast to oral administration, serum vitamin E levels rose and peroxide haemolysis fell to normal within 24 hours in all three children (Fig. 2). The vitamin E levels in M.L. and P.H. were greater after 24 hours than we have observed in any other

children. In M.T., who received the smallest loading dose, serum vitamin E and peroxide haemolysis became abnormal again after eight days, but in the other two children (who received much larger loading doses) the results of both tests were still normal after 12 and 17 days. In P.H., in whom biliary obstruction was already improving, serum vitamin E was still within the normal range 52 days after the load.

3 LONG-TERM ORAL ADMINISTRATION OF VITAMIN E

The response to the load tests had suggested a severe defect in vitamin E absorption, and in order to further investigate the severity of the malabsorption longitudinal studies were performed in 10 of the 17 children.

Effect of long-term vitamin E therapy in persistent obstructive jaundice

Each of the six children with persistent obstructive jaundice received very large daily doses (30-266 mg/kg body weight/day) of either the fat-soluble or water-miscible preparation of vitamin E for periods ranging from two months to three years. Despite such large doses serum levels of vitamin E remained at, or fell below, the low pretreatment values in all cases, and except for two of the children peroxide haemolysis remained essentially unchanged.

Studies during improvement of biliary obstruction

In S.G. vitamin E was started (40 mg/kg/day of the fat-soluble preparation) at a time when liver function tests were rapidly improving. Initially serum vitamin E was undetectable but after two weeks levels became normal and continued to rise during the remainder of the four months of treatment; levels remained normal after discontinuing the vitamin E.

In P.N. obstructive jaundice was associated with widespread skin xanthomata. Treatment with cholestyramine resulted in a fall in serum cholesterol and resolution of the xanthomata. Liver function has slowly improved. The water-miscible preparation of vitamin E, in a dose of 66 mg/kg/day, was started at the same time as cholestyramine when serum vitamin E was $0.29 \text{ mg}/100 \text{ ml}$ and peroxide haemolysis 98.2%. After 25 months of therapy, serum vitamin E had increased to $1.33 \text{ mg}/100 \text{ ml}$ and peroxide haemolysis had returned to normal (3.2%).

In P.H. signs of obstructive jaundice began to improve coincident with the intramuscular injection of vitamin E. In this patient serum vitamin E levels remained normal for at least 52 days (Fig. 2). The fourth child (J.G.) received no vitamin E supplement and, although serum vitamin E was not determined, disappearance of signs of biliary obstruction

Fig. 2 Intramuscular loads of alpha-tocopheryl acetate in three children with biliary obstruction.

was followed by a return of the increased peroxide haemolysis (60%) to normal.

Discussion

The degree of vitamin E deficiency found in the children with biliary obstruction is more severe than that associated with other malabsorptive states such as cystic fibrosis and coeliac disease (Muller and Harries, 1969), and we have only encountered lower levels of serum vitamin E in the rare condition of abetalipoproteinaemia (Muller, Harries, and Lloyd, 1970). Our findings in obstructive jaundice agree with those of Nitowsky *et al* (1956b), who found serum vitamin E to be 0.09 mg/100 ml in one infant with congenital biliary atresia and were unable to detect any vitamin E in the serum of another. In adults Woodruff (1956) was also unable to detect serum vitamin E in one patient with xanthomatous biliary cirrhosis; Klatskin (1954), however, in a study of 38 patients with obstructive jaundice, reported mean levels of 0.75 mg/100 ml $\pm$ 0.35 compared with normal values of 1.22 mg/100 ml $\pm$ 0.35. It is likely that the degree of vitamin E deficiency seen in patients with obstructive jaundice partly depends on the adequacy of body stores before

the onset of biliary obstruction. In children biliary obstruction is often present from birth or early infancy (as was the case in all our patients) when, even in normal babies, serum vitamin E levels are substantially less than in the older child or adult (Nitowsky *et al*, 1956a). It is therefore not surprising that vitamin E deficiency is more severe in babies with obstructive jaundice than in adults.

This study provides only indirect information on the absorption of pharmacological doses of vitamin E, and the results do not necessarily have any bearing on the role of bile salts in the absorption of physiological doses of vitamin E. The persistence, however, of very low levels of serum vitamin E, despite oral administration of large doses of alpha-tocopheryl acetate given for up to three years, suggests a severe defect in the mechanisms normally responsible for absorption of vitamin E. The fall in red cell haemolysis that occurred in two children after eight and 11 months of treatment, however, suggests that some of the vitamin was absorbed. Woodruff (1956) and Nitowsky *et al* (1956a) also reported a poor response to large doses of vitamin E in their patients. The rapid return of serum vitamin E and haemolysis to normal levels following intramuscular loads provides further evidence that the

deficiency was caused by intestinal malabsorption. Similar observations were made in one infant studied by Nitowsky *et al* (1956a). The fall in serum vitamin E and rise in peroxide haemolysis in two children two to three weeks after levels had been corrected by large intramuscular injections of vitamin E suggests that even with such doses repletion of body stores was not achieved.

In children with obstructive jaundice caused by conditions such as biliary atresia or 'neonatal hepatitis' flow of bile is almost completely obstructed (Strandvik and Norman, 1970), and the role of biliary obstruction in causing vitamin E malabsorption is demonstrated in our study by the two children who received treatment at a time when biliary obstruction was improving and responded well to much smaller oral doses of vitamin E than those given to the children with persistent obstruction.

Recent studies in man (Blomstrand and Forsgren, 1968; McMahon and Neale, 1970) and in experimental animals (Gallo-Torres, 1970) have suggested that bile plays a major role in the intestinal absorption of vitamin E. The precise mechanism by which bile influences the absorption of vitamin E is not clear. Bile salt micelles have an important role in the solubilization and absorption of dietary lipids (Hofmann and Borgstrom, 1962) and it is likely that such non-polar substances as the fat-soluble vitamins are particularly dependent on this solubilizing system (Dawson, 1967; Badley, 1970). This concept is supported by the observations of McMahon and Thompson (1970) who found vitamin E to be more efficiently absorbed from a mixed micellar solution than from an emulsion in the experimental animal. In children with extrahepatic biliary atresia Ricour and Rey (1970) have shown that the micellar phase in the small intestine is practically non-existent. It might be anticipated that in patients with biliary obstruction the water-miscible preparation of vitamin E by being less dependent on the solubilizing properties of bile salt micelles would be more efficiently absorbed than the fat-soluble preparations, as is the case in children with cystic fibrosis (Harries and Muller, 1971). The results of the present study, however, provide no evidence for this, and probably indicate that the water-miscible preparation failed to enhance intraluminal micelle formation.

Feldman, Salvino, and Gibaldi (1970) have shown that bile salts may alter the permeability of cell membranes and suggest that the transport of certain substances across gastrointestinal membranes may be influenced by such a mechanism. As a lipid antioxidant vitamin E may play an important role in maintaining the integrity of lipid-rich structures

such as cell membranes and it is possible that prolonged vitamin E deficiency could also affect the movement of substances across membranes. Gallo-Torres (1970) speculated that pancreatic juice contains an enzyme, alpha-tocopherol ester hydrolase, which is necessary for the hydrolysis of tocopherol esters before their absorption from the intestinal lumen, and that the presence of bile salts is necessary for this process. It is unlikely that the severe malabsorption observed in our patients was due to impaired hydrolysis of alpha-tocopheryl acetate since Woodruff (1965) reported a similar degree of malabsorption in an adult who received large doses of free tocopherol. We are not aware of pancreatic function tests being reported in children with biliary obstruction, and in the majority of adults with cirrhosis pancreatic insufficiency is probably caused by a direct action of alcohol (Badley *et al*, 1970). Although pancreatic function was not investigated in the present study, it is unlikely that pancreatic insufficiency was an important factor in causing malabsorption of the tocopherol esters since we have found much smaller doses (1 mg/kg/day) of the same preparation to be relatively well absorbed in children with cystic fibrosis (Harries and Muller, 1971).

Although defective absorption appears to be the main reason for vitamin E deficiency in obstructive jaundice other pathophysiological mechanisms may play a part. The major carrier of vitamin E in the blood is beta lipoprotein (McCormick, Cornwell and Brown, 1960; Pelkonen, 1963; Kayden, Silber, and Kossmann, 1965), and it might be anticipated that reduced levels of beta lipoprotein would be accompanied by a fall in serum vitamin E; in abetalipoproteinaemia, lack of the carrier lipoprotein is undoubtedly partly responsible for the severe deficiency in these patients (Kayden *et al*, 1965; Muller *et al*, 1970). In adults with cirrhosis Kater *et al* (1970) found a good correlation between the serum concentration of beta lipoprotein and vitamin E and considered that low levels of vitamin E could be accounted for by deficiency of the carrier lipoprotein. In their study, however, vitamin E absorption was not investigated and neither is it stated whether biliary obstruction was present in any of the patients. In many patients with obstructive jaundice beta lipoprotein concentrations are increased (Seidel, Alaupovic, and Furman, 1969), although in the terminal stage of hepatocellular failure defective synthesis may cause a fall in serum levels. Studies of serum lipoproteins in five of our patients with persistent obstructive jaundice (Lloyd, personal communication) did not show any reduction in beta lipoprotein concentrations, and the high serum concentration of vitamin E with prompt correction of peroxide haemolysis achieved after

the intramuscular loads suggests that the lipoprotein carrier was also functionally normal.

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