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# Vitamin E Deficiency in Man:

## BIOCHEMICAL EVIDENCE IN A PATIENT WITH XANTHOMATOUS BILIARY CIRRHOSIS

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No clinical syndrome directly attributable to a deficiency of vitamin E has been recognized in the human, although low levels of tocopherol in the blood have been noted in sprue[1] and microscopic pigmentation of the muscles has been recorded in this disease.[2,3] The specific biochemical and pathologic changes in experimental animals depleted of this vitamin are well documented. This is a report of several of the biochemical changes characteristic of experimental avitaminosis E which have been observed in a human patient; furthermore, the reversal of some of them following tocopherol therapy has been demonstrated.

### METHODS OF STUDY

The tocopherol nutriture of the patient here reported has been evaluated as follows: serum total tocopherol levels were determined by the micromethod of Quaife, Scrimshaw, and Lowry;[4] urinary creatine and creatinine were determined by standard methods; urinary pentose determinations were performed by the method of Minot, Frank, and Dziewiatowski[5]† hydrogen peroxide hemolysis of red cells was performed according to the procedure outlined by Rose and György[6,7] as modified by Gordon;[8] the assay of tocopherol in subcutaneous tissue was made as described by Quaife and Dju.[9]

### CASE REPORT

Mrs. E. A., aged 42 years, was first admitted to Vanderbilt University Hospital‡ in October, 1950, with xanthomatous biliary cirrhosis. She had had jaundice, light colored stools, dark urine, pruritus, and four to six bulky stools daily for five years. Four years before admission another clinic had recorded the findings of an enlarged liver, generalized xanthomatous skin lesions, and a serum cholesterol of 1332 mg per 100 ml. She had been placed on a low fat diet to which she adhered conscientiously. Three years before admission she fractured her left tibia and ambulation was rarely attempted after that time because of weakness. During the few months preceding her admission the weakness increased and abdominal distention was noted. She had had a simple hysterectomy three years previously.

Examination revealed a slightly emaciated

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† The analyses were kindly performed by Dr. Ann S. Minot.

‡ This patient was seen initially at the request of Dr. Ralph Larsen.

woman of 42 years whose skin and sclerae were olive-colored. Numerous xanthomatous plaques were widely distributed over her skin. The liver was not palpable but the spleen was enlarged to below the level of the umbilicus. The abdomen was distended with gas and fluid. There was no peripheral edema. The physical examination was otherwise within normal limits. Pertinent laboratory findings are summarized in Table I. Roentgen examination revealed generalized osteoporosis, and healed fractures of the left tibia and the eleventh dorsal vertebra.

Absorption of fat was studied by giving a test meal of 650 ml of homogenized milk containing 200,000 IU of vitamin A and 600 mg $\alpha$-tocopherol. The resulting chylomicron counts* and vitamin A and tocopherol levels indicate impairment of absorption (Fig. 1). Glucose and vitamin A, in a water-dispersible preparation, appeared to be absorbed normally. The addition of bile salts or emulsification in Tween 80®† had no influence upon the absorption of vitamins A and E from oily preparations.

The absence of measurable tocopherols in the serum was associated with creatinuria and pentosuria as indicated in Figure 2. The patient was given 100 mg of $\alpha$-tocopherol emulsified in Tween 80 daily. Her low fat, high protein diet was continued. In August, 1951, tocopherol medication was replaced by a placebo of Tween 80 in water. In May, 1951, severe low back pain was present on changing posture and three more compression fractures of lumbar vertebrae were found. In January, 1952, a pathologic fracture of the right femur occurred which healed following immobilization.

In May, 1952, the patient was admitted for further study. Pathologic fractures of several vertebrae evidently occurred during the movement associated with roentgen examination. The intravenous administration of calcium gluconate[10] was associated with a marked initial decrease in urinary phosphorus excretion, followed by an abrupt rise in serum phosphorus and a fall in serum calcium concentrations. This was regarded as evidence of intense compensatory activity upon the part of the parathyroid glands compatible with deficient calcium absorption.* A muscle biopsy was performed. Biochemical tests to evaluate vitamin E nutriture were repeated as indicated in Figure 2. On June 5th, the patient received 250 mg of tocopherol in the form of *d*-$\alpha$-tocopheryl polyethylene glycol 1000 succinate (tocopheryl PGS)† intramuscularly as a 20

TABLE I

Representative Laboratory Determinations

| | Date | | |
|---|---|---|---|
| | 10/30/50 | 5/26/52 | 4/17/54 |
| Hemoglobin—g/100 ml | 13.0 | 10.6 | 11.0 |
| White blood cell count per cu mm | 4,500 | 3,200 | 4,400 |
| Prothrombin time—% of normal | 63 | 76 | 49 |
| Total serum protein—g/100 ml | 5.5 | 6.4 | 5.5 |
| Serum albumin—g/100 ml | 2.6 | 2.8 | 1.8 |
| Cephalin flocculation at 48 hours | 4+ | 4+ | 4+ |
| Thymol turbidity—units | 20.0 | 7.5 | 11.0 |
| Direct serum bilirubin—mg/100 ml | 9.8 | 12.2 | 14.3 |
| Total serum bilirubin—mg/100 ml | 17.1 | 20.5 | 24.3 |
| Serum cholesterol—mg/100 ml | 220 | 155 | 130 |
| Serum calcium—mg/100 ml | 8.5 | 8.1 | 8.2 |
| Serum phosphorus—mg/100 ml | 2.9 | 2.5 | 3.4 |
| Serum alkaline phosphatase—Bodansky units | 10.0 | 15.5 | 17.3 |
| Serum vitamin A—IU/100 ml | 66 | — | — |
| Serum carotene—$\mu$g/100 ml | 18 | — | — |
| Nonprotein nitrogen—mg/100 ml | 26 | 36 | 24 |

* The chylomicron counts were made by Dr. Carl Anderson.

† These emulsions were prepared by Dr. H. C. Meng.

* Dr. Beverly T. Towery's aid in investigating and treating the osteomalacia is gratefully appreciated.

† This preparation was supplied by Dr. Philip L. Harris of Distillation Products Industries.

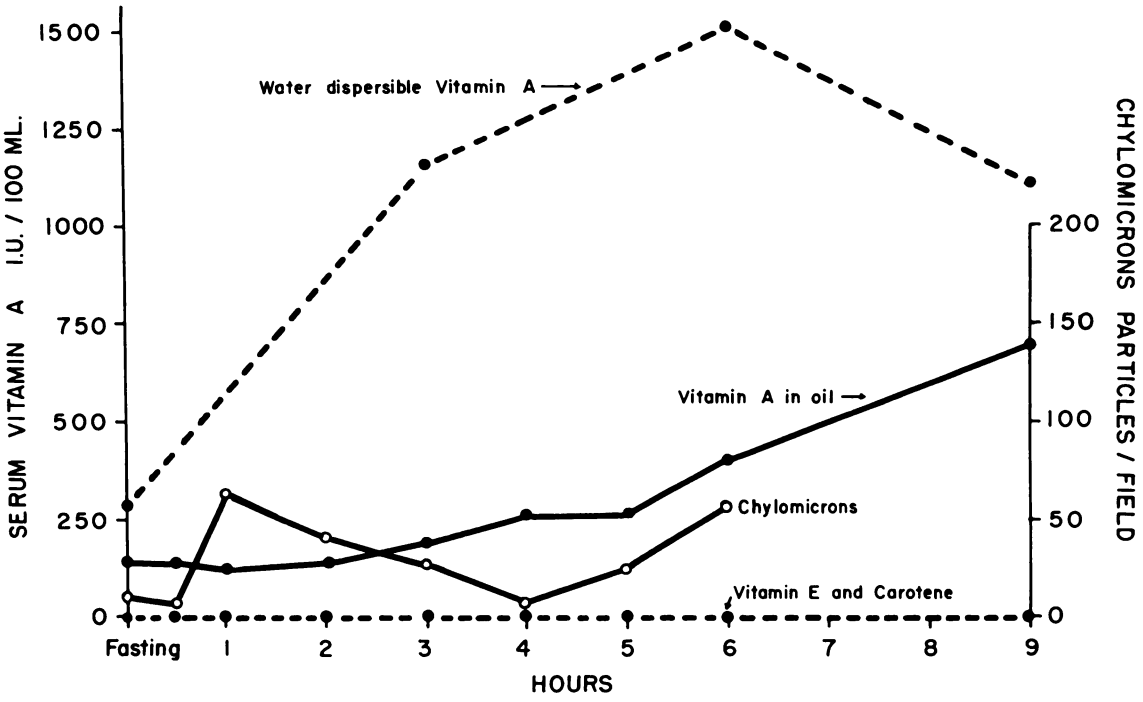


Fig. 1. The serum levels of vitamin A, total tocopherols, carotene and chylomicron counts following a test meal of 650 ml of homogenized milk fortified with 200,000 IU of vitamin A and 600 mg of $\alpha$-tocopherol. Both carotene and tocopherol levels were 0 in all the determinations. The initial high vitamin A level was due to several days' treatment with a water-dispersible preparation prior to this test. The absorption curve following the administration of 200,000 IU of vitamin A in water-dispersible form (Vi-Penta®-Roche) was performed two days later.

per cent solution in water. Two days later she received 42 ml of the same preparation orally, containing the equivalent of 2.1 grams of $\alpha$-tocopherol. Massive vitamin D therapy and supplementary calcium were also added to the treatment regimen.

In April, 1954, the patient was readmitted with ascites, pleural effusion, and dependent edema secondary to hypoalbuminemia. The skin xanthomata appeared to be regressing. Bleeding of the gums followed slight trauma. A softening similar to infantile craniotabes was felt on firm pressure over the tibia.

On October 20, 1954, death occurred in another hospital nine days following splenectomy. An autopsy* confirmed the clinical diagnosis of xanthomatous biliary cirrhosis.

* The autopsy was performed by Dr. Chester K. Jones of Jackson, Tenn.

## DISCUSSION

As has been predicted,[11] the possible occurrence of signs of vitamin E deficiency in the human might be expected in patients with severe defects in fat absorption who have been treated with a low fat diet for prolonged periods. The present patient had had a significant defect in fat absorption for at least five years prior to the present observations and had been on a low fat diet for over three years. Steatorrhea had been present during most of this period. When first seen, the absence of tocopherols in the blood serum suggested further investigation. In our experience, such extremely low levels of serum tocopherol are rarely found even in patients with sprue.[1] No tocopherol appeared in the serum following a test dose of 600 mg, even when administered with bile salts or when given with the surfactant, Tween 80.

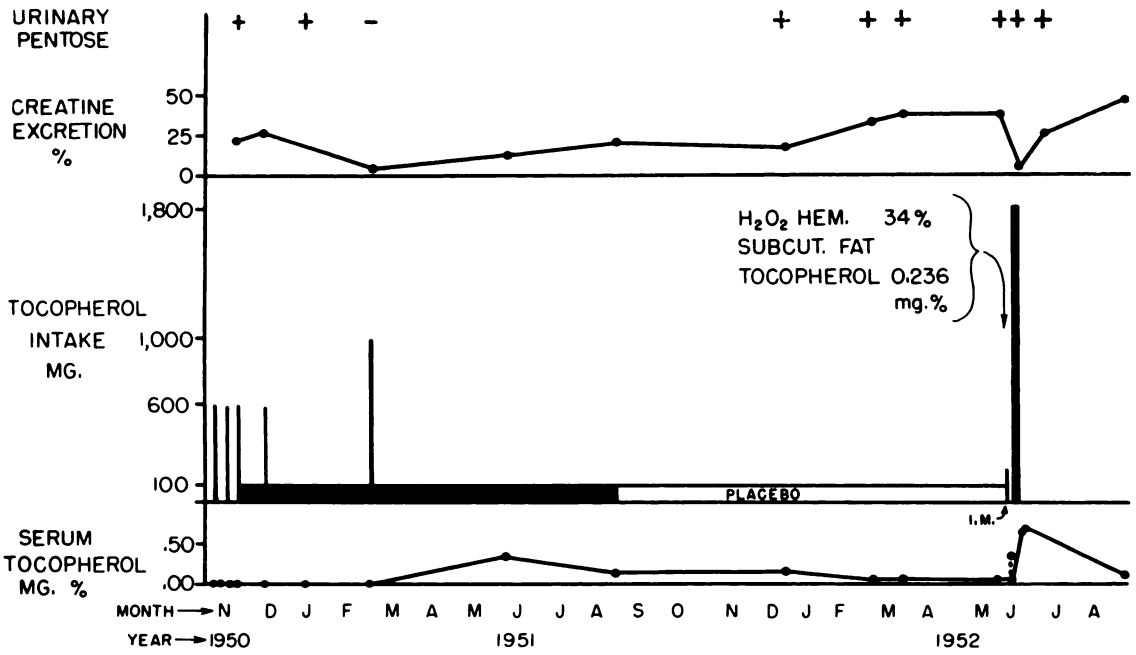


Fig. 2. Serial observations of pentosuria, creatinuria, serum total tocopherol concentrations and doses of tocopherol administered to this patient over a two-year period.

The levels of other fat-soluble vitamins were low and presumably they were poorly absorbed. The initial concentration of vitamin A in the serum was at the lower limits of normal. Although detectable amounts of carotenoids were present in the serum initially, subsequent observation usually failed to detect the presence of carotenoid pigments. The decrease in prothrombin could have resulted from either defective absorption of vitamin K or from liver disease. Because of the severity of the patient's illness, vitamin K therapy was not withheld in order to seek a further fall in prothrombin, but she had observed repeatedly that the bleeding from her gums was controlled by vitamin K administration. Her severe osteomalacia was probably due to reduced calcium absorption. Pathologic fractures ceased to occur following therapy with calcium and vitamin D.

The biochemical findings related to vitamin E nutriture are presented in Figure 2. At the onset, a creatinuria of about 25 per cent of the total creatinine, the presence of a pentose complex in the urine, and the absence of tocopherol in the serum characterized this biochemical picture. After three months of treatment with 100 mg of $\alpha$-tocopherol daily, in addition to the larger amounts administered for the determination of tocopherol absorption, the creatinuria and pentosuria disappeared. At this time no change in the serum tocopherol followed the administration of 1000 mg of $\alpha$-tocopherol as an absorption test. After another three months of therapy the serum level had risen to 0.35 mg per 100 ml.

During the nine months following replacement of the tocopherol preparation by a placebo, the pentosuria and creatinuria reappeared and the serum tocopherol level had fallen to 0.05 mg per 100 ml. Hydrogen peroxide hemolysis of red cells (34 per cent) was definitely increased. The muscle biopsy taken at this time showed extreme muscular atrophy without leukocytic infiltration or deposition of ceroid pigment. The biopsy could not be interpreted as characteristic of vitamin E deficiency as seen in the experimental animal. A specimen of subcutaneous fat removed at this time had a tocopherol concentration of 0.236 mg per

100 g.* This is extremely low, usual values for this age group averaging about 9 mg per 100 g.[12]

Because of the difficulty in absorbing fat-soluble preparations of vitamin E, the patient was treated with water-soluble tocopheryl PGS. The intramuscular administration of a single injection of 250 mg of $\alpha$-tocopherol in this form was not followed by a significant increase in the blood values. Two days later she was given 2.1 g of $\alpha$-tocopherol in the same form orally. This produced a rise in the serum total tocopherol to 0.33 mg per 100 ml after nine hours, and to 0.62 mg per 100 ml after four days of therapy. This regimen was followed by the disappearance of creatinuria, although the pentose complex continued to be present in the urine. Unfortunately, subsequent determinations of the peroxide hemolysis of the red cells were not considered reliable. Because of extensive pathologic fractures and the bed-ridden state of the patient, it was impossible to make any objective observations concerning muscular strength.

Recent observations by Nitowsky, Gordon, and Tildon[13] in infants with cystic fibrosis of the pancreas and biliary atresia demonstrate a similar correlation of defects in fat absorption with low serum tocopherol concentrations, creatinuria, and decreased resistance to peroxide hemolysis reversible by vitamin E therapy. Their report would suggest that states of poor vitamin E nutriture may occur rather frequently in patients having defective fat absorption. The patients reported by Albright and Stewart[15] and by Cooley and Hartman,[14] for example, have much in common with the present case. This case has been presented with the hope that it will stimulate others to enlarge upon these limited but suggestive observations.

## SUMMARY

The association of creatinuria and pentosuria with virtual absence of tocopherols from the blood of a patient with a defect in fat absorption subsisting on a low fat diet for several years has been reported. Increased susceptibility of the red blood cells to hydrogen peroxide hemolysis and an extremely low concentration of tocopherol in the subcutaneous fat were also found. Tocopherol therapy was associated with reduction of creatinuria on two occasions and with disappearance of the pentosuria on one occasion. It is suggested that these biochemical changes represent a deficiency of vitamin E in this patient.

## ACKNOWLEDGMENT

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* This analysis was performed by Dr. Dju through the courtesy of Dr. Karl Mason.

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### *Curiosity, Competition, and Faith*

"We seldom stop to think of why we do what we do; in fact, we seldom think philosophically at all. Many a research program is conducted in which the researcher has no clear idea as to just what he is trying to find out or what it would mean if he found it. Men work for years without stopping to examine critically the relevance of their efforts. Curiosity is often the underlying motivation, and not a bad one. It was probably responsible for the discovery by Prometheus of how to make a fire. Emulation is often clearly present, especially when a great scientist is surrounded by disciples. The competitive spirit, fortunately now on a more friendly basis than in the past, has a strong part in scientific motivation. It has become less artificial and less acrimonious as caste distinctions and false pride have lost their hold and as the stuffed shirts among us have dwindled almost to extinction. And the sporting instinct, whatever that is, which causes men to pit themselves against great odds, to revel in trial and adversity, to breathe the stimulating air of self justification in success, no doubt is often present. We do what we do for many unavowed reasons, and seldom pause to analyze them. But when we do reflect, we find that our primary motivations in scientific effort extend far beyond our casual and momentary reasons, even beyond the thought that what we do may, in its small way, benefit the human race in its struggle to control its environment and itself in the grim days that are sure to come.

For the scientist lives by faith quite as much as the man of deep religious convictions. He operates on faith because he can operate in no other way. His dependence on the principle of causality is an act of aith in a principle unproved and unprovable. Yet he builds on it all his reasoning in regard to nature."

—Vannevar Bush. *Report of the President of the Carnegie Institution of Washington*, December 9, 1955, p. 6.