STUDIES OF TOCOPHEROL DEFICIENCY IN INFANTS AND CHILDREN

V. An Interim Summary

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Vitamin E was recognized some 35 years ago as a fat-soluble substance necessary for reproduction in the rat. Its potency is measured by assay for fertility and its synonym, tocopherol, comes from Greek words which mean “to bear offspring.” Review of the original studies of Mason and his co-workers and of his interpretive writings provides a good stimulus for pediatric interest in the subject. It is proposed to review some literature on the pathologic lesions produced in animals and on the tocopherol content of foods, and then summarize data collected at the Colorado General, Sinai and Johns Hopkins Hospitals on tocopherol deficiency in infants and children. Most of the latter data and detailed references to the literature have been published elsewhere.

PATHOLOGIC FINDINGS IN EXPERIMENTAL ANIMALS

Although vitamin E has been dubbed the anti-sterility vitamin, its absence from the diet has produced a variety of pathologic states, differing from one species to another, and at different ages in the same species. Some of the conditions found are: Fetal resorption; testicular degeneration; encephalomalacia; “exudative diathesis”; generalized edema; brownish discoloration of smooth muscle, adipose tissue and liver; acute hemorrhagic necrosis of the liver; degeneration of renal tubules; focal necrosis of cardiac muscle; and nutritional muscular dystrophy.

Provocative findings in E-deficient animals that call to mind clinical problems in premature infants are: Hemorrhagic manifestations in rat fetuses and chick embryos; hemorrhages in the lungs, visceral and cranial cavities in puppies; subcutaneous, pulmonary and cerebral edema in young chickens; anemia in monkeys; and hemolysis after administration of large doses of vitamin K to rats.

The lesion which appears in the greatest number of species is nutritional muscular dystrophy, originally produced by dietary means in 1931, but not established conclusively as due to lack of vitamin E until 1939. It may occur as an acute, explosive process in young animals, the prototype being the “late lactation” paralysis of rats or as a chronic dystrophy in older animals. The following animals have shown one or both forms of the disease: Monkey, dog, rat, mouse, hamster, mink, rabbit, guinea pig, sheep, goat, cow, horse, pig, chick, duck, turkey, pheasant, guppy fish. Affected muscles are pale and gritty and show in varying degree interstitial edema, leukocytic infiltration, fragmentation of muscle fibers with loss of striation, nuclear breakdown, hyaline necrosis and calcification, fibrosis, deposition of acid-fast pigment, and proliferation of sarcolemma nuclei. If a prolonged E deficiency can be maintained,
the lesions may resemble those of human progressive muscular dystrophy, adipose and connective tissue replacing muscle fibers. However, extravagant claims for benefit in muscular dystrophy or clinical conditions resembling some of the experimental states have exploded quickly, and thus vitamin E has become a shady lady to be approached gingerly by respectable or discreet investigators in human nutrition.

Although the primary mechanism of action of tocopherol in the living organism is still not defined, many of its effects have been attributed to its role as an intracellular anti-oxidant. This does not preclude a more specific action of vitamin E through some enzyme system, as for example in the enzymatic reduction of cytochrome C by reduced diphosphopyridinenucleotide.

TOCOPHEROL CONTENT OF FOOD
Quaife, Harris and their co-workers developed methods of chemical assay for tocopherol, which have permitted analyses of foods, tissues and blood. They have found that the richest dietary sources of vitamin E are vegetable oils like those obtained from corn, soya bean, peanut, coconut or cottonseed, with cereal products and eggs next in order of nutritional importance. Other vegetables supply little of the daily intake of vitamin E, both because of their low concentration of total tocopherols and because only a small proportion may be alpha-tocopherol with its relatively greater biologic activity. Animal products have a low content of total tocopherol, but a high proportion is the alpha form. The value of cereal products as a source depends on the extent of the milling process. The mean daily intakes of total and alpha-tocopherol have been estimated as 24 and 14 mg per day when calculated from figures for average food consumption. Human colostrum has been found to contain from 0.13 to 3.6 mg/100 ml; in human milk frozen within a week of collection, the values ranged from 0.10 to 0.48 mg/100 ml. The mean concentration of 0.24 mg/100 ml is approximately twice that of evaporated milk, diluted 1:1 with water.

STUDIES OF HEMOLYSIS OF ERYTHROCYTES AND PLASMA TOCOPHEROL IN INFANTS AND CHILDREN
Our first studies were of the susceptibility of erythrocytes to hemolysis by hydrogen peroxide, a technique for detecting tocopherol deficiency, details of which were graciously made available to us by György and co-workers prior to publication. It is seen in Figure 1 that whereas the mean hemolysis for four groups totaling 131 adults ranged from 2 to 7%, the means for the 349 newborn infants were from 34 to 53%. There were no significant differences between full-term and premature infants when allowance was made for the economic status of the mother. Administration of tocopherol uniformly reversed this in-vitro hemolysis.*

When simultaneous measurements of tocopherol in the plasma and hemolysis were made, it was found that appreciable hemolysis was absent in 119 (95%) of 125 subjects in whom the tocopherol in the plasma was above 0.5 mg/100 ml. This was in accord with in-vitro studies and the studies by J. MacKenzie of premature infants fed supplements of alpha-tocopherol acetate. Below 0.5 mg/100 ml, statistically-significant negative correlations of from −0.49 to −0.7 were found in subjects other than newborn infants; in the latter, susceptibility of erythrocytes to hemolysins in hydrogen peroxide was found to be related to erythrocyte catalase activity as well as to concentration of tocopherol in the plasma.

In Figure 2 is presented a summary of the results of 577 determinations of tocopherol in the plasma in subjects of different ages, none of whom had received supplements of tocopherol. It is seen that a group of 25 normal, adult, hospital personnel had a mean tocopherol concentration of 0.84 mg/100 ml, and that a group of post-partum

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* The tocopherol or esters used in these studies were supplied by the Hofmann-LaRoche Company, Nutley, New Jersey; the Distillation Products Industries, Rochester, New York; and the U. S. Vitamin Corporation, New York, New York.
HEMOLYSIS IN HYDROGEN PEROXIDE
COMPARISON OF RESULTS FOR ADULTS AND UNFED NEWBORN INFANTS

Fig. 1. The percent hemolysis is the ratio of hemoglobin liberated from aliquots of erythrocytes incubated with hydrogen peroxide and diluted with a buffer to that liberated by dilution in distilled water.

mothers had a higher mean concentration of 1.32 mg/100 ml. In contrast to this elevated concentration in maternal blood (previously reported by others and described teleologically as preparation for lactation) newborn infants, full-term and premature, had mean concentrations of only 0.23 or 0.26 mg/100 ml, varying from one-fourth to one-seventh

PLASMA TOCOPHEROL LEVELS - SUMMARY OF 577 DETERMINATIONS

Fig. 2. No dietary surveys were made for these subjects. The premature infants received for the most part, partially skimmed cows' milk, and some of the infants over 30 days of age received oral iron medication, which oxidizes tocopherol. The full-term infants, with the exception of one group on human milk, as well as the subjects with cystic fibrosis of the pancreas and biliary atresia, received diets of cows' milk plus varied solids.
of the maternal concentrations in simultaneous determinations. These findings confirm the reports of other observers on concentrations of tocopherol in plasma and tissue and indicate that tocopherol does not cross the placenta easily.

Full-term infants fed artificially showed a gradual rise in plasma tocopherol, the mean of 0.33 mg/100 ml at 7 weeks being significantly higher than that in the newborn period. In the group of infants 2 to 5 months of age, the mean is still higher, 0.42 mg/100 ml with a further rise to 0.58 for subjects 6 to 24 months and 0.73 for children 2 to 12 years. As far as superficial histories could determine, none of these subjects had received tocopherol supplements, and the lack of popularity of tocopherol leads us to believe no supplementation had taken place. In contrast with this slow rise in artificially fed, full-term infants, the small number of breast-fed infants showed a mean of 0.72 mg/100 ml at average age of 7 weeks. Wright, Filer and Mason reported that this rise in breast-fed babies actually takes place within 6 days after birth.

In the premature infants fed partially-skimmed cows' milk for the most part, the plasma tocopherol showed a decrease rather than a rise over the 2-month period studied. This was to be expected from the studies of hemolysis in peroxide, and confirmed the findings of Wright and his co-workers. Administration of tocopheryl esters regularly raised the concentration of tocopherol in the plasma.

In patients with biliary atresia and cystic fibrosis of the pancreas, plasma concentrations of tocopherol averaged only 0.11 and 0.15 mg/100 ml. These results confirmed and extended the reports of others and were also to be expected from previously reported data on hemolysis of erythrocytes.

Of what significance are these findings of low concentration of tocopherol in the plasma of newborn babies, in premature infants fed cows' milk, and in children with steatorrhea? And what does it mean that plasma tocopherol rises, to the concentration found in adults, with breast feeding in a matter of days or weeks and with artificial feedings in a matter of months or years? In general one does not wish to equate an extracellular concentration with either stores or intracellular metabolic effects—the limitations are obvious whether one considers ascorbic acid or potassium or tocopherol.

Depletion studies are one method of attempting more precise correlations. In adults, Horwitt and co-workers showed that more than a year of tocopherol depletion is necessary before plasma tocopherol decreased from average concentrations of 1.0 to 0.5 mg/100 ml. A plateau of at least another 12 months at 0.5 mg was found during which body stores, presumably in adipose tissue, yielded tocopherol to maintain the plasma concentration. Furthermore since tocopherol and cholesterol concentrations in the plasma vary directly, one must consider that in the newborn infant, for example, the factors responsible for the increase in cholesterol and certain lipoproteins may also be partly responsible for the increase in tocopherol.

On the other hand, the low concentration of tocopherol in the tissues of fetuses and young infants, the finding of positive hemolysis tests with hydrogen-peroxide in young infants shortly after they are placed on skimmed milk, the prompt reversal of the persistently low concentration of tocopherol in the plasma and positive hemolysis tests in premature infants and subjects with steatorrhea by administration of tocopheryl esters, all indicate that young infants (particularly prematurely-born ones), and subjects with prolonged steatorrhea are candidates for clinical manifestations of tocopherol deficiency.

ADDITIONAL STUDIES IN PATIENTS WITH STEATORRHEA

Patients with cystic fibrosis of the pancreas or congenital biliary atresia have a prolonged absorptive defect and therefore were particularly worthy of further study. Since many of these patients have flabby muscles, we considered the possibility that this might represent evidence of specific
tocopherol deficiency rather than of general malnutrition. We chose as a basis for our first studies the finding that rabbits on E-deficient diets showed creatinuria before clinical signs of weakness developed, and that this could be abolished by administration of tocopherol.25

We have to date studied the creatine excretion of 17 children with cystic fibrosis of the pancreas while receiving creatine-poor diets before and after tocopherol administration. In 13, data for 6 of whom have been published, there was an appreciable initial creatinuria, and this diminished markedly in 10 of 11 subjects when tocopherol was given in doses which raised the concentration of tocopherol in the plasma to levels which prevented hemolysis of erythrocytes in hydrogen peroxide. In four subjects, for reasons not clear, there was virtually no initial creatinuria even though plasma tocopherol was low and hemolysis in hydrogen peroxide significant.

We have as yet no objective evidence of clinical improvement after administration of tocopherol to these patients. Mothers as well as personal physicians have reported general improvement in some instances, but the parents' quiet desperation which leads them to an unusual degree of cooperation, the variability of a disease with a course which depends so much on the associated pulmonary infection, and the difficulty in quantitating muscle power make critical clinical evaluation hazardous.

In four infants with biliary atresia, significant creatinuria has also been present. The response of the plasma tocopherol of patients with biliary atresia to oral administration of tocopherol esters, even in water soluble form, has been irregular; when given intravenously in doses sufficient to raise plasma tocopherol and reverse hydrogen peroxide hemolysis, it has been without effect on the creatinuria. It may be that the route of administration of tocopherol is important.25 Woodruff28 reported an adult with biliary cirrhosis, low plasma tocopherol and pentosuria; raising the plasma tocopherol produced a decrease in creatinuria in two, and of pentosuria in one of two trials.

In an attempt to find a pathologic correlate of this biochemical evidence of tocopherol deficiency in patients with steatorrhea, Oppenheimer29 reviewed the necropsy findings in 48 patients with cystic fibrosis of the pancreas at Johns Hopkins Hospital. Since there are differences in the extent to which the dystrophic process affects different muscles or even different regions of any one muscle,25 it was unfortunate that only small portions of muscle were available for study and these in only 10 patients. Six of these patients were less than 6 months of age but one of the four remaining subjects, an infant of 24 months, showed, in addition to rickets and metaplasia of bronchial epithelium (presumably due to deficiencies of vitamins D and A), focal areas of muscle necrosis, hyalinization and infiltration of leukocytes—lesions similar to those of nutritional muscular dystrophy caused by vitamin E deficiency in other species.

Since that report, Weinberg made an extensive post-mortem examination of the muscles of an infant with congenital biliary atresia who died in Sinai Hospital at 15 months of age. The patient while alive had a low plasma tocopherol and increased hemolysis of erythrocytes in hydrogen peroxide. The muscles showed, in addition to marked atrophy, numerous foci of degeneration with loss of striation, hyalinization, necrosis, proliferation of sarcolemma muscle cells and round cell infiltration—lesions similar to those described in Oppenheimer's patient and in animals with vitamin E deficiency. In another 15-month-old infant with congenital biliary atresia, examined extensively by Oppenheimer at Johns Hopkins Hospital, no lesions have been found.40

In a patient with sprue reported in 1908,41 suggestive lesions of the muscle are described and it has been suggested25 that the areas of hyalinization described by Blackfan and Wolbach in the muscles of vitamin A-deficient infants may have been due to an associated vitamin E deficiency.

Stowens42 has been unable to find lesions...
suggesting vitamin E deficiency in specimens of muscle submitted to the Pediatric Registry at the Armed Forces Institute of Pathology. An average of two specimens of muscle were submitted from 170 cases, and an average of six specimens were available from 50 additional cases of congenital biliary atresia in the Institute's files. Since the muscle lesion is spotty and possibly infrequent, extensive examination of muscle from additional patients with steatorrhea is indicated.

The failure of intravenously administered tocopherol to affect creatinuria in patients with biliary atresia, the absence of lesions in the cited case studied extensively, as well as in the 220 cases studied less so, call attention to the report by MacKenzie25 that in the rat the extent of the lesions in the muscle is increased when tocopherol deficiency is combined with deficiency of pyridoxine, vitamin A or protein. Young, Dinning and Day43 have also reported an interrelationship between pyridoxine and tocopherol.

SHOULD INFANTS AND CHILDREN RECEIVE SUPPLEMENTS OF TOCOPHEROL?

An attempt to answer this difficult question leads to consideration of the criteria used for other vitamins.44 The answer is obviously an affirmative when one considers therapy for overt, specific-deficiency syndromes; examples in infancy and childhood are: Scurvy, rickets, beriberi, pellagra, megaloblastic anemia, ariboflavinosis, and convulsions due to pyridoxine deficiency. A deficiency may develop as a result of: a) inadequate intake, e.g., scurvy; b) excessive loss, e.g., xerophthalmia and night blindness associated with steatorrhea; or c) a defect in utilization, e.g., vitamin D-resistant rickets. In some syndromes, biochemical evidence of deficiency occurring before overt symptoms is accepted as indication for general preventive supplementation. For example, the hydroxyphenyluria of premature infants, and decreased serum phosphate and citrate, are taken as indications for early general use of vitamin supplements of C and D, respectively.

Controversy arises, however, when one enters the field of supplementation designed to offer widespread prevention in the general population. Judgment is made difficult by economic factors, such as the cost to manufacturers of enrichment of foods or replacement of losses due to processing. The Council on Food and Nutrition of the American Medical Association and the Food and Nutrition Board of the National Research Council have adopted a policy recommending enrichment of certain processed foods with supplements to levels sufficient to prevent deficiencies, or in the case of some foods to restore the initial or natural levels. An example of benefit is the addition of vitamin D to evaporated milk so that one quart of the reconstituted milk supplies 400 I.U. of vitamin D. The competition between manufacturers of vitamin products, and "cultural" factors which decide not only the length of our automobiles but also the supplements for our infants' diets, are added hurdles for the physician.

In the case of tocopherol, the investigations cited justify Goldsmith's recent statement that vitamin E may be coming of age in human nutrition. Our current thinking about supplementation may be summarized as follows:

1) Patients with cystic fibrosis of the pancreas should be given supplements of tocopherol. They have low concentrations of tocopherol in the plasma, increased hemolysis of erythrocytes by hydrogen peroxide, and an excessive creatinuria which has responded to administration of tocopherol in a manner like that of animals with nutritional muscular dystrophy. Lesions similar to those produced in animals on E-deficient diets have been found in one case of cystic fibrosis of the pancreas, and "ceroid" pigment (a manifestation of E deficiency occurring particularly when unsaturated fats are in the diet) has been reported in the intestinal walls of patients with cystic fibrosis of the pancreas.45 Clinically, many of the patients have flabby
muscles, but there is as yet no objective evidence for the impression of clinical improvement following therapy with tocopherol. The daily administration of 200 to 300 mg of water-miscible tocopheryl esters has regularly led to an elevation of plasma tocopherol to concentrations above 0.5 mg/100 ml, and a reversal of the tendency to hemolysis, but we have had no opportunity as yet to determine the minimal effective dose, a matter of economic import.

2) Patients with congenital biliary atresia also have a prolonged steatorrhea and biochemical manifestations of tocopherol deficiency, i.e., excessive creatinuria, low plasma tocopherol, increased hemolysis in hydrogen peroxide. Oral administration of water-miscible tocopheryl esters is only irregularly effective in raising plasma tocopherol, and the intravenous administration does not decrease creatinuria although it does raise plasma tocopherol. Whether the route of administration or the advanced cirrhosis affect utilization is not clear. Lesions resembling those of nutritional muscular dystrophy have been found in one of two cases studied extensively, but not in 220 cases where only small amounts of muscle were available for examination. Because of the poor nutritional status and prognosis of these patients, tocopherol supplementation appears indicated. Studies are needed to determine the conditions under which effective absorption of oral supplements can be obtained.

3) Normal full-term infants are born with low tissue stores of tocopherol, low plasma concentrations and a susceptibility of erythrocytes to hemolysis in peroxide. Because the plasma tocopherol rises with unsupplemented diets, and a concomitant fall of in vitro hemolysis occurs, and because there is no evidence as yet of clinical or biochemical aberrations referable to the low stores, there appears to be no reason for supplementing the diets of normal full-term infants with tocopherol. This is certainly true for the breast-fed baby.

4) Premature infants are born with low tissue stores and low plasma concentrations of tocopherol, and the plasma concentrations fall even lower and susceptibility of erythrocytes to hemolysis persists, with partially skimmed cows' milk feedings. Because the tocopherol content of whole cows' milk is low, because storage of human milk leads to a loss of tocopherol, because many premature infants have a defect in fat absorption, it is likely, although not yet shown, that premature infants fed more varied diets as they grow older will lag behind full-term infants in adequacy of tocopherol. In view of their rapid rate of growth, and in the absence of known toxic effects, small supplements of tocopherol are perhaps indicated for premature infants. Although we have hesitated to make this recommendation, while we search for clinical, physiologic or biochemical correlates of deficiency, we believe the recommendation may turn out to be correct. The occurrence of hemorrhagic manifestations in E-deficient rat fetuses, chick embryos and newborn puppies are sufficiently suggestive of troublesome clinical problems in the early newborn period to make this phase of the subject particularly worthy of intensive study.

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LABORATORY DIAGNOSIS OF CONGENITAL GALACTOSAEMIA AT BIRTH, B. Schwartz et al. (Lancet, 1:24, January 4, 1958.)

Although it is impractical to apply the test described in this report to all newborn infants in order to detect sporadic cases of galactosaemia, it would be useful to employ it with newborn infants born into families known to be afflicted with this disorder. The test is based on determination of the enzymic activity which is responsible for normal metabolism of galactose-1-phosphate. Individuals who develop galactosaemia are deficient in this enzyme in the erythrocytes. Blood is obtained from the umbilical cord of the newborn infant and the erythrocytes are incubated in a system containing galactose. In the erythrocytes of individuals who would develop galactosaemia if fed the lactose in milk, incubation of the erythrocytes with galactose leads to accumulation of galactose-1-phosphate in the cells. Details of the method are provided and its application to diagnosis of galactosaemia in a newborn is described. With this information in hand a galactose-free diet can be prescribed and the damaging effect of extraneous galactose avoided.
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