TOCOPHEROL DEFICIENCY IN INFANTS AND CHILDREN WITH STEATORRHEA

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It is the purpose of this paper to describe the current status of our studies in infants and children with cystic fibrosis of the pancreas or biliary atresia. Our interest in tocopherol deficiency dates from the suggestion by the Owens in 1949 that premature infants might have such a deficiency because of their faulty absorption of fat and the use of partially skimmed cows’ milk mixtures, low in vitamin E, to circumvent this handicap. The finding by György, Cogan and Rose that the hemolysis of red blood cells in hydrogen peroxide could be used as a measure of tocopherol deficiency in newborn infants led to studies, begun at the University of Colorado in 1951 and continued for the past five years at the Sinai and Johns Hopkins Hospitals. We found that the red blood cells of premature infants were hemolysed by dilute solutions of hydrogen peroxide and that this could be regularly prevented by administration of tocopheryl esters to the infants. It was subsequently found that positive hydrogen peroxide hemolysis tests and low plasma tocopherol concentrations occurred as frequently in newly born full-term as in premature infants, and that their persistence could be related inversely to the tocopherol content of the diet. We therefore turned our attention to patients with malabsorption of fat such as is associated with cystic fibrosis of the pancreas, biliary atresia and celiac syndrome.

In twenty-one patients with these diseases, hemolysis over 50 per cent was found in 79 per cent of thirty-eight observations. In twenty-one observations on patients with other diseases associated with severe malnutrition, only two observations on an eczematous infant whose diet had been restricted to cereal and water for an appreciable period showed hemolysis over 50 per cent. That this high incidence of markedly positive hemolysis tests in children with steatorrhea was due to tocopherol deficiency was indicated by reversal of the tests by administration of tocopheryl esters either orally or intravenously. It was further found that the average plasma

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This investigation was supported in part by a grant (A-494 C2) from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, United States Public Health Service.
tocopherol was low in these children, confirming the findings by Darby and his coworkers in adults with sprue,7 and by Filer and his coworkers in children with cystic fibrosis of the pancreas or celiac syndrome.8 To date we have made eighty-five determinations of plasma tocopherol in twenty-nine children with cystic fibrosis of the pancreas, twenty-two observations in eight infants with biliary atresia and forty observations in forty hospital “normals” as controls. The mean plasma tocopherol levels in the patients with steatorrhea are only 1/5–1/6 of that of the hospital “normals” (Table I).

We were left then with conclusions that one might have forecast, namely that patients with steatorrhea had a lowered plasma concentration of a fat soluble vitamin, tocopherol, and as a reflection of this, an increased tendency of their red cells to hemolyze when incubated with hydrogen peroxide. Since there is no evidence that, under customary circumstances, free peroxides are liberated in vivo, the hemolysis in hydrogen peroxide could only be considered as an inverse measure of tocopherol concentration, and in the present state of our ignorance, of no other obvious biologic significance.

We turned then to a search for possible clinical, biochemical, physiologic or pathologic correlates of this deficiency, and wish to express our gratitude to Dr. Karl E. Mason, Professor of Anatomy at the University of Rochester for the stimulation we have obtained both from his original studies and from his writings. In 1942, he pointed up changing concepts of the role of vitamin E in animal nutrition.9 Originally dubbed the anti-sterility vitamin, because of its ability to prevent fetal resorption in the rat on an E deficient diet, its lack has been associated with a variety of lesions in different species. Among these are testicular degeneration, hemorrhages in embryos and fetuses, nutritional encephalomalacia, a generalized edema called “exudative diathesis”, brownish discoloration of smooth muscle, adipose tissue and liver, acute hemorrhagic necrosis of liver, degeneration of renal tubules, focal necrosis and fibrosis of cardiac muscle, and
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nutritional muscular dystrophy. And of these, the latter, described initially by Goetsch and Pappenheimer, is the most common lesion, occurring either as an acute explosive process, the prototype of which is the lactation paralysis of rats, or as a chronic dystrophy in older animals. Species affected by one, or both forms of the disease are rats, mice, guinea pigs, hamsters, rabbits, mink, dogs, pigs, lambs, calves, foals, goats, chicks, ducks, turkeys, pheasants, guppy fish and monkeys. Affected muscles are pale and gritty and show in varying degrees interstitial edema, leucocytic infiltration, fragmentation of muscle fibers, with loss of striation, nuclear breakdown, hyaline necrosis and calcification, fibrosis, deposition of acid-fast pigment, and on occasion proliferation of sarcolemma nuclei and regeneration.

Since patients with cystic fibrosis of the pancreas, biliary atresia, and celiac syndrome have flabby muscles, it occurred to us that this might represent evidence of specific tocopherol deficiency rather than of general malnutrition. We chose as a basis for our first studies the findings by MacKenzie and McCollum that rabbits on tocopherol deficient diets showed creatinuria before clinical signs of weakness developed, and that this could be abolished by administration of tocopherol.

We have to date studied the creatine excretion of eleven children with cystic fibrosis of the pancreas while on creatine-poor diets before and after tocopherol administration. In eight, data for five of whom have been published, there was an appreciable initial creatinuria which diminished markedly when tocopherol was given in sufficiently large doses to raise the plasma tocopherol to the levels found in normal children. In three, there was virtually no initial creatinuria even though plasma tocopherol was low and hydrogen peroxide hemolysis high.

The diminution in creatinuria noted after tocopherol could have been due to (a) diminished synthesis by the liver and kidney (b) increased deposition in or diminished extrusion from muscle or (c) increased renal tubular reabsorption of creatine. If the effect of tocopherol were to diminish creatinuria through an effect on the renal tubules, one might expect plasma creatine either to rise or show no change. In each of four subjects studied to date, however, tocopherol administration has led to a drop in plasma creatine. In Figure I are presented data collected from P.W., a three year old boy with cystic fibrosis of the pancreas, studied at the University of Maryland through the courtesy of Dr. Samuel Bessman. It is seen that the oral administration of 500 mgm of tocopherol daily led to an increase of plasma tocopherol from 0.1 to 1.8 mgm per cent by the seventh day of tocopherol administration and a concomitant drop in hemolysis of red cells. Over a twenty-one day period, urinary creatine while on a creatine-poor diet dropped from 48 to 14 per cent of the total
creatinine-incomplete collection of specimens prevents expression of the results as a creatine coefficient, i.e. in mgm per Kg per day. What is most interesting is the finding that this diminished creatinuria occurred while the plasma creatine was dropping from 1.3 mgm per cent to only 0.54 mgm per cent, suggesting that the effect of tocopherol was prerenal, either on the liver or muscles. Studies using the more precise calculations of creatine clearance after intravenous administration of creatine loads are continuing.

In addition to this biochemical correlate of tocopherol deficiency, there are certain pathologic findings to report. Dr. Ella Oppenheimer has reviewed the post-mortem findings in a group of forty-eight patients with cystic fibrosis. Unfortunately, only small portions of muscle were available for study and these in only ten patients. Six of these were less than six months of age at the time of death, but one of the four remaining subjects, an infant of twenty-four months, showed in addition to rickets and metaplasia of bronchial epithelium, presumably due to deficiencies of vitamin D and A, focal areas of muscle necrosis, hyalinization and infiltration of leucocytes, similar to the lesions of vitamin E deficiency in other species.

**Fig. 1.** The effect of tocopherol administration on plasma tocopherol, plasma creatine, urinary creatine-creatinine ratio and hydrogen peroxide hemolysis of erythrocytes of a child with cystic fibrosis of the pancreas.
We should like to come now to the infants with biliary atresia. We have studied eight infants all of whom show low plasma tocopherols and markedly positive hemolysis tests. Attempts to reverse these findings have required much large oral doses of tocopheryl esters than in the patients with cystic fibrosis, suggesting that their tocopherol deficiency is severe. It was therefore at first disappointing to note in two patients that intravenous tocopheryl esters failed to decrease creatinuria even though plasma tocopherol had been raised markedly. We considered the possibility that the route of administration, or cirrhosis of the liver already present in these patients, might interfere with utilization of tocopherol by muscle. We propose to investigate this because we have recently found the typical lesions of nutritional muscular dystrophy in an infant with congenital absence of the bile ducts who died at age fifteen months. This patient, who will be reported in detail elsewhere, had been jaundiced since birth and at age six months had an exploratory laparotomy which revealed no external bile ducts. At age three months, before attempts to supplement her diet with tocopheryl esters, plasma tocopherol was 0.31 and 0.36 mgm per cent and hydrogen peroxide hemolysis 57 to 94 per cent. During a three week period of oral supplementation with two different preparations, plasma tocopherol rose on one occasion to 0.51 mgm and then decreased to 0.14 mgm per cent. Hemolysis in hydrogen peroxide remained above 50 per cent for most of this period. Because of the irregular effects of the supplements, they were discontinued, and at age fifteen months, when she died with pneumonia and evidence of severe wasting, plasma tocopherol was zero.

An extensive examination of the muscles was made, a procedure which is for obvious reasons not customary. In addition to marked atrophy, the muscles showed numerous foci with loss of striation, hyalinization, necrosis, calcification and leucocytic infiltration, lesions very similar to those described in Oppenheimer’s patient and in animals on vitamin E deficiency.

A more careful pathological examination of the muscles in additional patients with steatorrhea is obviously indicated. Since the lesion is usually spotty we do not believe that histological examinations of biopsies will necessarily be rewarding, but we are making chemical examinations of biopsies before and after tocopherol treatment. Major Daniel Stowens, director of the registry of pediatric pathology of the American Academy of Pediatrics and Dr. Richard Follis, director of the section on nutritional pathology at the Armed Forces Institute of Pathology have offered to try to obtain from pathologists adequate specimens of muscle at autopsies of patients dying with steatorrhea.

We have not yet determined whether there are any physiologic or clinical correlates of the vitamin E deficiency in these patients. We have begun
studies of muscle power of both normal children and children with steatorrhea. Mothers as well as personal physicians of some of the patients have reported marked general improvement of some of the patients with cystic fibrosis of the pancreas after tocopherol. The quiet desperation of the parents, occasionally banded into clubs, plus the variability of a disease whose course depends so much on the associated pulmonary infection, makes clinical evaluation difficult.

In summary, we believe that infants and children with cystic fibrosis of the pancreas or biliary atresia have a deficiency of tocopherol, presumably because of faulty absorption of this fat soluble vitamin. The evidence for this is (a) the finding of low plasma tocopherol and increased tendency of red blood cells to hemolyze in hydrogen peroxide (b) the reversal of these findings by administration of tocopheryl esters either orally or intravenously (c) the finding in patients with cystic fibrosis of the pancreas that tocopherol decreases creatinuria, apparently as a pre-renal effect and (d) the finding in one infant with cystic fibrosis of the pancreas and in one infant with biliary atresia of lesions in the muscle which are similar to those of nutritional muscular dystrophy seen in animals on vitamin E deficient diets.

Search continues for clinical and physiologic correlates of the biochemical and pathologic findings.

REFERENCES

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DISCUSSION

Dr. Howard Burnham Sprague (Boston): In view of the localized, but rather violent, espousal of vitamin E in certain cardiac conditions, was there any observance of an effect on cardiac muscle?

Dr. George D. Gammon (Philadelphia): I would like to ask a question. I believe Schoenheimer's work on turnover of creatine in muscles shows about 2 per cent per day, and, presumably, if one repaired the defect in muscle, the effect would be a reduction of creatine excretion and an elevation, or at least a stabilization of creatininuria.

I noticed in the patient on the slide there, although his creatine output dropped, his creatine co-efficient indeed did not rise, but, in fact, fell off a little.

Has that been true of all your cases? Is this assumption correct, that if you repair the muscle you have more functioning muscle, and hence a larger creatinine excretion?

Dr. Ham: We followed this hemolysis test with interest and worried about it. I wonder if you would comment on it and if you realize it is a vital enigma to the originator of the test and wonder if you have any further conclusions as to the mechanism.

Dr. Gordon (closing): Dr. Sprague, we have no observations on cardiac muscle. Because of the "violent espousal" you have already mentioned, we were a little reluctant to point out that in one patient there were some electrocardiographic changes that disappeared after tocopherol.

Dr. Gammon, in the patient that we demonstrated, the creatinine coefficient decreased from 26 to 22 milligrams. In other patients, creatinine coefficients have for the most part stayed the same.

The point to which you refer is of course correct, that with marked wasting of muscle, such as occurs in more extensive muscular disease, creatinine coefficients have decreased as creatine coefficients have increased. We do not know the explanation for the failure of the urinary creatinine to increase except that possibly we are dealing with a spotty rather than an extensive disease.

I think it is important to recognize that it takes time for signs of deficiency to develop. The monkeys studied by Dr. Karl Mason in Rochester and Drs. Day and Dinning in Arkansas, were on an E deficient diet for as long as two or three years before muscular dystrophy developed.
Patients studied by Dr. Horwit at Elgin State Hospital, have been on a diet with virtually no E for two years before the tocopherol level decreased to five-tenths of a milligram, the level below which we have regularly observed hemolysis of red cells in hydrogen peroxide.

In answer to Dr. Ham's question with regard to the hemolysis test, originally our concern was entirely with this and why the red blood cells hemolyze. Unfortunately, we have been distracted by the patients with cystic fibrosis of the pancreas.

On the basis of the effectiveness of a variety of substances other than tocopherol as inhibitors of hemolysis Dr. Nitowsky has suggested that perhaps tocopherol inhibits a lipoxidase which acts on unsaturated fatty acids in the envelope of the red cell and thus protects against hydrogen peroxide hemolysis. This mechanism has been suggested independently by others.

We know of no biologic significance for the test. We haven't given up our interest and may get back to these studies.

There is no evidence that free peroxides are liberated in normal metabolism. It has been suggested that they are after radiation injury. It has occurred to us that in the newborn infant where hemoglobin oxygen saturation rises within a matter of minutes, sometimes from 50 to 95 %, there may be free peroxide liberated and maybe this is what starts the hemolysis that takes place in newborns. Dr. Eugene Kaplan, a very competent investigator in hematology has joined us and proposes to study this problem since all newborn babies have low plasma tocopherol.