SPECIAL ARTICLES

THE DEFECT IN UTILIZATION OF TOCOPHEROL IN PROGRESSIVE MUSCULAR DYSTROPHY[1, 2, 3]

EXPERIMENTAL observations on 15 patients with progressive muscular dystrophy have indicated that this condition closely resembles the muscular dystrophy induced in animals by deprivation of tocopherol. In experimental muscular dystrophy in animals, tocopherol given by mouth promptly reduces the creatinuria and restores muscular function, whereas the parenteral administration of the vitamin (as the free substance) is practically without effect, suggesting that tocopherol undergoes some necessary change in the gastrointestinal tract. Tocopherol (either the free substance or the acetate, phosphate and succinate esters) in oral doses of from 1 to 5 g was without effect on the creatinuria of any of the 15 patients except one in whom the dystrophic process was of unusually slow progression. However, tocopherol that had been incubated in the stomach of a normal man lowered the creatine output of about one half of the subjects. The biologically active substance could not be removed from the gastric expression by extraction with ethyl ether. The *in vitro* incubation of the vitamin with an extract of hog stomach and duodenum rendered tocopherol effective in reducing creatinuria in patients with slowly progressing symptoms when propylene glycol was added to the incubation mixture, but no effect on creatinuria was observed when choline or ethanolamine was substituted for the propylene glycol. In one mild case tocopherol given together with the extract of hog stomach and duodenum lowered the creatine output.

Cold-pressed wheat-germ oil produced no demonstrable change in the excretion of creatine, but wheat-germ oil obtained by extraction with warm ethylene dichloride reduced the creatinuria of 4 patients. Similar effects were observed in 2 other subjects with Graves' disease. The ethylene dichloride extract of ether-defatted wheat germ[4] yielded a residue that was about 15 times as effective as the extracted oil of whole wheat germ.

Tocopherol treated by the following procedures was effective in about 60 per cent. of the patients, the effect being definite in mild cases and minimal or absent in severe cases: (1) refluxing on the steam bath with ethylene dichloride, ethylene dibromide, ethylene chlorohydrin or ethylene bromohydrin with constant stirring in an atmosphere of nitrogen; (2) refluxing with ethylene dichloride in the presence of ascorbic acid as an antioxidant; (3) heating with ethylene oxide in the autoclave at 200° in an atmosphere of nitrogen at a pressure of 2,000 pounds. The probability that the biologically active substance was a condensation product of ethylene glycol and tocopherol in ether linkage was suggested by the previous work of Renshaw,[5] John,[6] Fernholz,[7] Marle[8] and Smith[9] who had prepared the ethylene glycol and other ethers of hydroquinone, duroquinone and phenol by methods similar to those used in the present investigation. The active principle was readily soluble in ether and only slightly soluble in water, although the product formed in the *in vivo* incubation experiments in the normal man was readily soluble in water.

On the basis of these findings and of observations made by numerous workers in experimental muscular dystrophy induced by tocopherol deprivation, it was postulated that the substance with which tocopherol forms a condensation product in the body should (1) be a glycol which could form an ether linkage with the free hydroxyl group of tocopherol, (2) contain several more hydroxyl groups than ethylene glycol for the final condensation product to be readily soluble in water, (3) be one that enters into reaction in the absorption of fatty acids, since the antagonistic effects of unsaturated fats on tocopherol appear not to be satisfactorily explained by the oxidative destruction of the vitamin and (4) be more easily available in the intestinal tract than are the short chain glycols. Moreover, a substance apparently so essential for the utilization of tocopherol would likely be available in the natural sources of tocopherol, for example, wheat germ.

One substance that seemed to satisfy these postulated requirements is inositol. Therefore, the following investigations were carried out: Equimolecular amounts of benzene hexachloride and alpha tocopherol in absolute alcohol containing KOH were refluxed on the steam bath with constant stirring in an atmosphere of nitrogen. The condensation product was readily soluble in water, and could be removed from ether solutions by extraction with water. Micro-

[1] Preliminary report.

[2] From the Departments of Medicine and Psychiatry, Cornell University Medical College, and the Russell Sage Institute of Pathology in Affiliation with The New York Hospital, New York.

[3] Aided by grants from the Nutrition Foundation, Inc., and the National Foundation for Infantile Paralysis, Inc.

[4] A. T. Milhorat, F. C. Weber and V. Toscani, *Proc. Soc. Exp. Biol. and Med.*, 43: 470, 1940; A. T. Milhorat, V. Toscani and W. E. Bartels, *Proc. Soc. Exp. Biol. and Med.* (in press).

[5] R. R. Renshaw and C. Y. Hopkins, *Jour. Am. Chem. Soc.*, 55: 1524, 1933.

[6] W. John, E. Dietzel and P. Gunther, *Zeits. Physiol. Chemie*, 252: 208, 1938.

[7] E. Fernholz and J. Finkelstein, *Jour. Am. Chem. Soc.*, 60: 2402, 1938.

[8] E. R. Marle, *Jour. Chem. Soc.*, 101: 305, 1912.

[9] R. A. Smith, *Jour. Am. Chem. Soc.*, 62: 994, 1940.

analyses, while not conclusive due to difficulty in removing the salts, suggested that the substance was a monoether of inositol and tocopherol. The material was stable in alkaline solution, but an acidity of pH 2.0 or lower split the substance and destroyed the activity on creatinuria. Direct comparisons can not be made but in rough figures, the substance was 2,500 times as effective as wheat germ oil obtained by ethylene dichloride extraction and 40,000 times as effective as wheat germ itself.

In one patient with muscular dystrophy of moderate severity, a single dose of 60 mg given with alkali reduced the creatinuria appreciably in 24 hours, and to one half the control level in 3 days. The excretion of creatine continued at this low level for a period of 8 days and then gradually rose to its previous control level. However, the effect was smaller and of shorter duration in 4 other patients with rapidly progressive symptoms. In one of these subjects a single dose of 95 mg lowered the creatine output only 14 per cent. for 3 days.

Although tocopherol or inositol given alone was without effect on creatinuria, definite effects were observed in 5 of 7 patients when these two substances were given together in equimolecular amounts for 1 or 2 days. The dosage of the mixture required to produce effects on creatinuria appeared to be proportional to the rate of progression of the dystrophic process. In general, the mixture was from 1/8 to 1/30 as effective as the condensation product. Incubation with an extract of hog stomach and duodenum increased the effect of the mixture of tocopherol and inositol on creatinuria; this effect was greater than that of equivalent amounts of tocopherol and propylene glycol similarly treated.

The observations suggest that tocopherol forms a condensation product with inositol in the gastrointestinal tract (tocopherol-inositol ether) and that the inherited defect in muscular dystrophy is a deficiency in this reaction of condensation. The degree of this deficiency appears to determine the rapidity with which muscular disability progresses. Patients in whom the disease process is mild can synthesize sufficient amounts of the condensation product when large amounts of both tocopherol and inositol are given together, but those in whom the disease is more rapidly progressive will probably require the condensation product itself.

A complete report with details of data and acknowledgments is in preparation. Investigations on the effect of prolonged administration of this product on clinical status in a large series of patients are in progress.

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MITOSIS IN REGENERATING LIVER[1]

IN one-month-old rats, the rate of mitosis is at a maximum 24 hours after partial hepatectomy.[2] The experiments described here were designed to determine whether this maximum rate could be further increased. All substances to be tested were administered intravenously 24 hours postoperatively and the remaining liver removed for assay 3 hours later. In each experiment 5 to 10 animals were given the test substance, while an equal number of controls received normal saline. Nuclei were isolated by the citric acid method and counts made with a hemocytometer.[3] For simplicity, only nuclei in metaphase and anaphase were classified as being in mitosis. Variations of 10 per cent. in the mitotic count were within the limits of error of the method and were therefore not considered significant.

Of 9 preparations of chromatin[4] from rat liver which were tested, 7 produced an increase in mitosis of 25 to 100 per cent., one showed an increase of only 12 per cent., while another gave a decrease of 13 per cent. Chromatin from beef liver gave an increase of 70 per cent., and of two preparations of rabbit liver chromatin tested, one increased the mitotic rate by 55 per cent., the other 290 per cent. Several preparations of chromatin made at room temperature all had no effect on mitosis.

When isolated chromatin was extracted with 1 M NaCl a considerable portion remained insoluble. Four preparations of this insoluble fraction were tested and none found to produce a significant change in the rate of mitosis. Of seven preparations of the soluble fraction, two gave increases of only 19 to 22 per cent., while the remainder showed increases of 60 to 140 per cent. When stored at 5 to 10° C, two of the active soluble fractions lost their stimulating effect in 2 to 4 days. One preparation of fat-free chromatin produced a 200 per cent. increase in mitotic rate.

The following substances were found to have either no effect or a negative one: Various crude fractions from the liver other than chromatin, casein digests (Stearn's Amino Acids, Amigen), l-cysteine, dl-methionine, insulin, adenosine triphosphate, adenylic acid, lecithin, biotin, lipid from chromatin.

The increase in the number of nuclei in metaphase and anaphase is not in itself sufficient evidence for an increase in the rate of mitosis, for such a result may be obtained if mitosis is arrested at either of these stages. Counts were therefore made of the relative

[1] The work described in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the University of California.

[2] A. Marshak and R. Byron, Jr., unpublished.

[3] A. Marshak, *Jour. Gen. Physiol.*, 25: 275–291, 1941.

[4] A. Claude and J. S. Potter, *Jour. Exp. Med.*, 77: 345–354, 1943.