Alpha-Tocohydroquinone and Muscle Dystrophy

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AS PART of a continuing clinical study of myopathies, α-tocohydroquinone was tested for possible therapeutic effectiveness in patients with muscular dystrophy. The rationale was based on the similarity of muscle histopathologic changes present in dystrophic patients and in laboratory animals with nutritional muscular dystrophy induced by α-tocopherol deficiency. These skeletal muscle lesions are characteristic of vitamin E deficiency, having been observed in more than 20 species of laboratory and farm animals. In these animals, the muscular dystrophy can be prevented by administration of α-tocopherol or certain of its oxidation products. In the case of human dystrophy, α-tocopherol therapy is ineffective. Patients with progressive muscular dystrophy usually have normal levels of α-tocopherol in their blood and tissues. They have no difficulty in absorbing, transporting, and storing α-tocopherol; however, it has been postulated that such individuals might lack the ability to metabolize it *per se*, and that this metabolic defect or block could be bypassed by administering a partially metabolized form of α-tocopherol.

Of the degradation products of α-tocopherol which might be a normal-occurring metabolite and which could be prepared in sufficient quantities for a clinical test, α-tocohydroquinone was a likely candidate (Fig. 1). Alpha-tocohydroquinone has been altered chemically from α-tocopherol enough to destroy antisterility activity but it is very active in preventing muscular dystrophy in rabbits, according to Mackenzie and Mackenzie.[1] West and Mason[2] found it effective also in preventing muscular dystrophy in the hamster. They have developed a sensitive bioassay for antidystrophy agents based upon the relative numbers of degenerating and regenerating muscle fibers in muscle sections taken from dystrophic hamsters after treatment for 10 days with graded doses of test material. Furthermore, Milhorat and co-workers[3] reported that α-tocohydroquinone reduced creatinuria in patients with progressive muscular dystrophy.

Consequently, a clinical trial was arranged to test the working hypothesis that by administering α-tocohydroquinone the metabolic block in α-tocopherol metabolism might be bypassed and muscle functions returned toward normal.

METHODS AND MATERIALS

Subjects

Ten boys and two girls, ranging in age from 6 to 14 years and, with one exception, manifesting various phases of the Duchenne type of muscular dystrophy,[4] were used as subjects. Seven were assigned to α-tocohydroquinone therapy, and five to placebo administration. Each patient was given at intervals a bottle of capsules identified only by his name. Which patients received the drug and which the placebo was not disclosed until the end of the test. Examinations were

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made at six-month periods. Therapy continued for 12 to 18 months.

Alpha-Tocohydroquinone

The α-tocohydroquinone was prepared* by oxidation of *d*-α-tocopherol to α-tocoquinone followed by reduction to the hydroquinone form as shown in Figure 1. Gelatin capsules

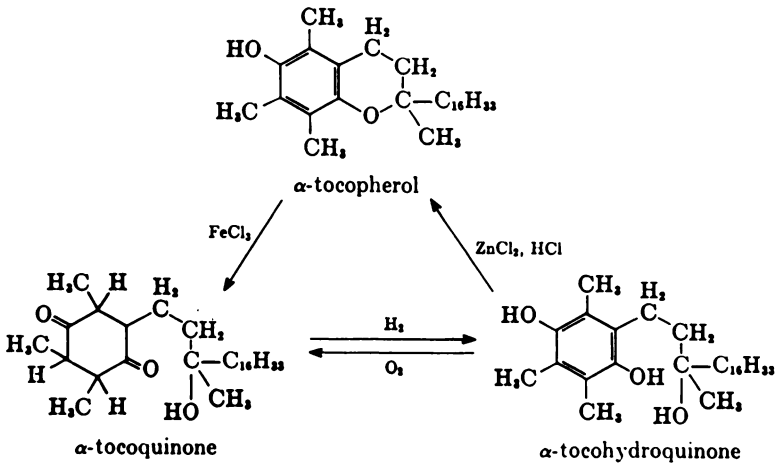

Figure 1

containing either 50 or 100 mg of the α-tocohydroquinone and placebos of identical appearance were made available for use.† Analysis of the capsules at intervals throughout the course of the test showed that the compound was completely stable.

Dosage

The dosage levels of α-tocohydroquinone varied from 200 to 600 mg daily and provided intakes for individual patients of approximately 7, 14, or 25 mg per kg body weight. The largest dose represented about one-half the dosage level, on a body weight basis, found to be minimum for curing experimental muscular dystrophy in vitamin E-deficient hamsters. In this connection, the data in Table I show the relative potency of α-tocopherol and various tocol derivatives as determined by hamster bioassay.[2] It is evident from the data in Table I that α-tocopheroxide, also a partially oxidized derivative of α-tocopherol, is about three times more effective than α-tocohydroquinone and deserves a clinical trial.

Efforts were made in two subjects to test the α-tocohydroquinone at levels of 50 mg/kg, which is commensurate with the effective dose in hamsters. However, a depression of prothrombin levels, which was not controlled by menadione (25 mg daily), necessitated return of the patients to the 25 mg/kg dose. The possibility of a prothrombin-lowering effect of α-tocohydroquinone in the patients had been anticipated, but not at levels as low as 50 mg/kg, as the result of the preliminary toxicity tests which had been carried out in rats. Massive doses (1000 mg/kg body wt.) had been found to cause internal hemorrhage, anemia, and death. Subsequent tests in which menadione was used at various levels as a protective anticoagulant showed that in the rat, vitamin K prevented the toxic manifestations resulting from large doses of α-tocohydroquinone and

TABLE I

Minimal Effective Dose for Repair of Dystrophy in the Hamster[2]

Substance	Amount
	mg
Alpha-tocopherol	2
Beta-tocopherol	4
Delta-tocopherol	>16
Alpha-tocoquinone	6
Alpha-tocohydroquinone	6
Alpha-tocopheroxide	2

* Distillation Products Industries, Rochester, N. Y.

† Eli Lilly & Co., Indianapolis, Ind.

that α-tocohydroquinone is, in fact, an antivitamin K. The antivitamin to vitamin ratio was determined to be about 1600 to 1 (700 to 1 on molar basis).

Criteria of Response

Five criteria were utilized in evaluating patient response to therapy: (1) creatine and creatinine excretion; (2) serum aldolase levels; (3) electrocardiograms; (4) motor-age muscle function tests; and (5) split-frame movies in Kodachrome, in which carefully planned sequences adapted to the motor limitations of the subject, and allocated to specified segments of 100-foot films, provided remarkably well synchronized recordings of motor activities prior to and after therapy. A device was inserted into the camera to mask the right half of the film during the filming of each subject as he performed exercises showing maximum muscular ability. After an interval of approximately six months, the same film was used (with the masking device reversed to protect the already exposed left half of the film) to photograph the same child performing the same exercise. The film was then developed and projected for evaluation. The left half of the picture on the projection screen showed the motor ability of the subject prior to, and the right half after, therapy. Slight changes in muscular ability could be observed by this technique.

RESULTS

In the dystrophic subjects, the degree of creatinuria increased quite markedly with increasing age and there was no appreciable change in the excretion of creatinine (Fig. 2). This is in contrast to values given for normal children of comparable ages[5] which show a slight decrease in creatinuria and a marked increase in creatinine excretion (Fig. 2). The ratio of creatine to creatinine excreted in the urine is a particularly good way of expressing this response since, as shown in Figure 3, children with muscular dystrophy are characterized by an increasing ratio with age, whereas the ratio for normal children actually decreases. Furthermore, only a single sample of urine, rather than a 24-hour specimen, is required for determination of creatine-creatinine ratio.

Serum aldolase levels were definitely above normal in all subjects (24 to 60 units compared to normal values of less than 10). This is in accord with the findings of Schapira and Dreyfus and their group.[6,7] However, the elevated values were not diminished by the

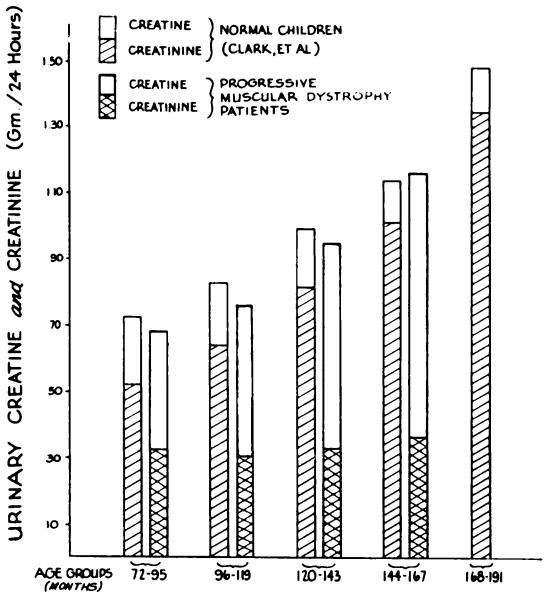

Figure 2

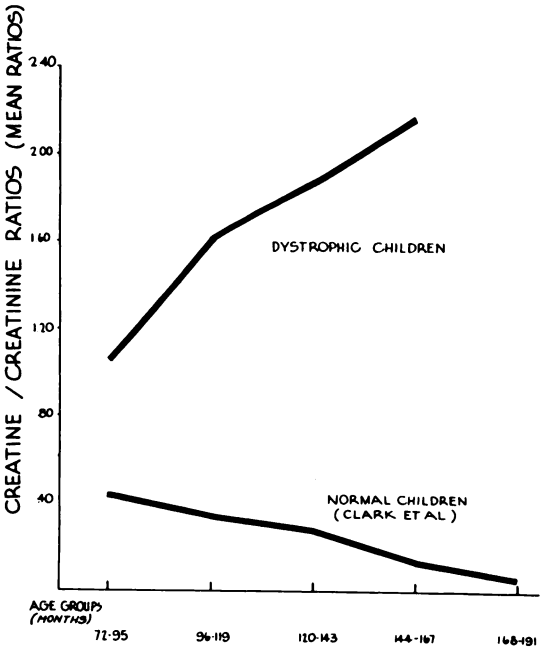

Figure 3

therapeutic measures employed in our subjects.

Motor-age muscle function tests, and routine testing of individual muscles and muscle groups, indicated no improvement with α-tocohydroquinone therapy over conditions existing prior to therapy. The electrocardiographic record showed no significant changes.

The recordings by split-frame cinephotography provided the most convincing and reliable evidence as to patient response, or lack of response, to therapy, and emphasized the merits of this technique in overcoming the fallacies of memory and in providing a permanent pictorial record for subsequent study and reference. In the case of patients maintained on therapeutic levels of 7 to 25 mg of α-tocohydroquinone daily, these records indicated a definite progress of the disease which, as far as can be determined, differed very little from that in the placebo group. Many of the sequences revealed unmistakable loss of muscle functions, sometimes combined with compensating attitudes. These losses were more evident in coarser movements of the extremities and trunk (walking, ascent and descent of stairs, bicycle riding, rolling over, crawling on hands and knees, going from prone to seated or to erect position, abduction at the shoulder joint, piling of blocks) than in activities requiring finer movements and co-ordination (picking up and placing of pegs in perforated board, writing, making of contacts for light bulb circuits).

SUMMARY

Alpha-tocohydroquinone at dosage levels of 7 to 25 mg/kg body weight for 12 to 18 months failed to influence the course of muscular dystrophy in children as measured by creatine excretion, serum aldolase activity, motor-age muscle function tests, and muscle function recorded by split-frame cinephotography.

Split-frame cinephotography has great merit in evaluating response, or lack of response, to drug therapy in progressive muscular dystrophy and in neuromuscular diseases in general. It also has great potential value in forming the basis of a permanent pictorial record of the course of progressive muscular dystrophy, and in analyzing the relative rate of loss of muscle function in different individuals suffering from the disease.

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DISCUSSION

Dr. C. Woodruff (Nashville, Tenn.): During the course of some 10 years that vitamin E levels in the serum have been done in the Division of Nutrition, we have determined vitamin E levels on about 175 patients that were seen in nutrition consultation. Among these were 28 patients who had serum tocopherol concentrations below 0.5 mg per 100 ml. A good many of these were cases of sprue that have been reported by Darby and Jones. There were four patients in this group who had practically no tocopherol in the blood. Two of these had celiac disease, one was a boy of 16 with Whipple's disease, and the last was a woman aged 42 years who had xanthomatous biliary cirrhosis of 10 years' duration. She had a severe defect in fat absorption, as manifested by steatorrhea, and a diminished ability

to absorb vitamin A. In conjunction with others at the Medical School, we have made some observations on this woman which are pertinent .

When she was first seen her serum tocopherol concentration was zero. No absorption followed the oral administration of 600 mg of α-tocopherol. Neither emulsification with Tween 80® nor the concurrent administration of bile salts resulted in the absorption of measurable amounts of tocopherol. She excreted creatine to the extent of 25 per cent of her total creatinine output. A pentose complex was present in her urine. She was treated with 100 mg of α-tocopherol daily emulsified with the aid of Tween 80. After three months of this therapy the creatinuria and pentosuria disappeared. After another three months of treatment the serum tocopherol level reached 0.34 mg per 100 ml. For the ensuing nine months she was given a placebo. The creatinuria and pentosuria reappeared and the serum tocopherol concentration fell to 0.06 mg. per 100 ml. Biopsy revealed a concentration of 0.236 mg. of tocopherol per 100 g of subcutaneous tissue, as determined in Dr. Karl Mason's laboratory. Hydrogen peroxide hemolysis of her red cells was increased at 34 per cent. She was given 250 mg of α-tocopherol intramuscularly in a water soluble form (d-α-tocopheryl polyethylene glycol 1000 succinate) supplied by Dr. Philip L. Harris. Since no increase was noted in the serum, she was given 2.1 g of α-tocopherol in the same form orally two days later. The serum concentration increased to 0.36 mg per 100 ml after six hours, and reached 0.64 mg per 100 ml after four days of such therapy. The creatinuria again disappeared, but the pentosuria continued to be present. The muscle biopsy made before this course of treatment showed extensive atrophy but the findings were not typical of avitaminosis E in the experimental animal and no "ceroid" pigment could be found. Because of her debilitated state no objective observations concerning her muscular strength could be made. These observations would suggest that at least biochemical evidence of vitamin E deficiency may be found in patients having severe defects in fat absorption of relatively long duration. (This case will be reported in detail in a future issue of this journal—Ed.)

DR. A. S. MINOT (Nashville, Tenn.): Ever since it has been shown by many investigators that muscular dystrophy can be produced in a wide variety of laboratory animals by withholding vitamin E from the diet, the idea has persisted in the minds of both clinicians and laboratory workers that there must be some basic fundamental common ground in clinical and vitamin E-deficient muscular dystrophy.

If you worked in this field as long ago as I did, you recall the heyday of clinical enthusiasm and hope which resulted when it was demonstrated that the various manifestations of nutritional dystrophy could be reversed by the administration of α-tocopherol. It then seemed reasonable to hope that the clinical condition might be equally successfully treated by such simple measures as the administration of various types of preparations of vitamin E. We need not rehearse the disappointments and failures which thus far have persisted throughout all attempts at influencing clinical dystrophy by simple therapy of this type.

In the papers we have heard here today the similarities between the two types of dystrophy have been adequately reviewed. The pictures we saw yesterday showing progressive muscular dysfunction in monkeys suffering from vitamin E deficiency were strikingly similar to the progressive deterioration seen in clinical cases of dystrophy. We have stressed also the histopathologic similarity of the changes in the muscles in the two types of dystrophy. The creatinuria which develops in the vitamin E-deficient animals and which has been discussed repeatedly as a useful criterion of the progress of the developing dystrophy is also characteristic of clinical dystrophy. Here, too, the progressively increasing weakness is accompanied by an increase in the urinary output of creatine.

There is another chemical analogy in the two conditions which has interested us here and which we reported in papers published some time ago. At the present time we do not know the significance of the observation.

We have, however, noted in some 20 or 30 clinical cases of muscular dystrophy that the urines consistently contain a reducing substance which is not fermentable and which is not one of the usual urinary constituents which may cause reductions with the usual sugar reagents. We finally succeeded in isolating this substance as an osazone which seems to meet the criteria of a pentosazone both as regards melting point and elementary analysis. Further studies with the help of Dr. Dziewiatkowski, who was here in the biochemistry department at that time, indicated that the reducing substance was probably being excreted as a pentose-phosphorus complex. The same material was also isolated from the urine of rabbits with vitamin E-deficient dystrophy. This is another superficial biochemical observation which is common to both types of dystrophy.

Then we come to the dissimilarities in the two conditions. Of course, the vitamin E-deficient animal has a very low serum tocopherol level. The tissues are depleted of vitamin E or tocopherol. This is in contrast to the fact that in clinical muscular dystrophy, as stated this morning, the levels of tocopherol are normal. In our series here we have found a range from about 0.7 to 1.2 mg of tocopherol per 100 ml of serum in untreated cases of clinical muscular dystrophy. Dr. Karl Mason and others have shown from studies of autopsy material from clinical cases that the tocopherol level in diseased muscles is as high or in some cases even higher than the levels found in normal muscles of persons of similar age. Then we have the tremendous differences in response to therapy. The vitamin E-deficient animal given adequate amounts of the vitamin promptly shows a decrease in the amount of creatine excreted and a gradual increase in muscle creatine as evidence of restoration of muscles. Less promptly, but gradually and eventually completely, the reducing substance we have described disappears from the urine of the treated animals as muscle function is restored. In marked contrast to this is the persistence of abnormal urinary findings and progressive deterioration of muscle function in cases of clinical dystrophy despite all our endeavours to furnish vitamin E in any form, or by any known route.

So if we continue to adhere to the hypothesis, and I think most of us do, that there is a common basic defect in the two conditions, there is a world of questions, of which you are much more aware that I am, which must be answered. In clinical dystrophy, is something blocking the utilization of, or in some way inactivating, the vitamin E which we find ready and waiting in dystrophic muscles? In our analyses of such muscles do we perhaps measure tocopherol which has been in some way altered so that it no longer functions as vitamin E? Of course, the wide variety of tocopherol compounds used in therapy represent attempts to circumvent any such possible inactivation.

We do not yet know the function of vitamin E in normal muscles. I think we suspect it is a necessary part of some enzyme system—perhaps a cofactor in one or several enzymes concerned with the metabolic release of energy for muscle contraction. In clinical dystrophy does vitamin E fail to get to, or to be built up into, these enzymes?

Progressive muscular dystrophy is often but not always congenital—I mean that while it is not always possible to obtain a familial history, in many instances you can. The disease is often listed as a congenital anomaly of metabolism. Is it beyond possibility that in the two types of dystrophy the defect is in the same enzyme system? May we not on the one hand have failure of the enzyme system because of lack of the necessary vitamin E, and, on the other hand, one due to a lack or defect in the apoenzyme of the same system? I am talking in a field that I know very little about, but it seems to me we should work very hard to find the answer to the fundamental question of what vitamin E does. I think we all have the feeling that if we ever find out the exact role of this vitamin in muscle metabolism we can then point our finger and say that this is the same step which is defective in clinical dystrophy. Whether it is defective for a reason that can be corrected, or whether the metabolic gap is one that we may hope to bridge by appropriate therapy, are questions we can't hope to answer until we know more about the gap we are trying to bridge.