dium heparin samples examined that met the USP potency requirement, 1 mg. of protamine sulfate neutralized from 87 to 122 sodium heparin USP units, a maximum variation of 42%.

Within each type of sodium heparin examined, an apparent correlation was observed between heparin units neutralized by 1 mg. protamine sulfate and heparin potency. No correlation was observed between sulfur content and the neutralization values.

## SUMMARY

Although all types of sodium heparin materials were not included, and no attempt was made to quantitate the parameters examined, namely tissue, species, potency, and process, this study established that there is a significant difference in the neutralization of different types of sodium heparin by protamine sulfate.

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# Hexahydrocoenzyme $Q_4$ in Pseudohypertrophic Muscular Dystrophy

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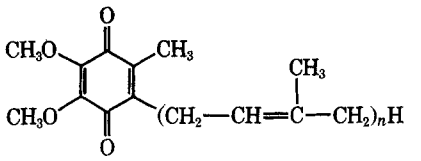
**Abstract** □ Hexahydrocoenzyme $Q_4$, 250 mg. per day for 8 months followed by 1000 mg. per day for 4 months, did not improve muscle strength or alter serum and urine creatine and creatinine, serum creatine phosphokinase, or aldolase, or a battery of other clinical and laboratory indexes including oral glucose tolerance and associated insulin, growth hormone, and inorganic phosphorus levels in 19 boys with pseudohypertrophic muscular dystrophy of the Duchenne type. The failure to observe beneficial or other changes during the ingestion of hexahydrocoenzyme $Q_4$ might have been because of: (*a*) the intrinsic inactivity of the substance in Duchenne dystrophy, and (*b*) the low dose level, particularly if this dystrophy should be one of the vitamin-dependent diseases of genetic nature which involves vitamins of both the water- and oil-soluble category. In any case, the effective dosage of hexahydrocoenzyme $Q_4$ for the genetic muscular dystrophy of mice in a previously reported study was approximately 10–50 times that used in this clinical study. The dosage for the mice was "massive" in terms of their body content of coenzyme Q. Hence, the studies herein reported do not exclude the possibility that higher homologs of the coenzyme Q group, *i.e.*, $Q_5$–$Q_{10}$, might have beneficial effects in human muscular dystrophy. In such trials, coenzyme $Q_{10}$ would certainly be the most important, since it is present in human tissues.

**Keyphrases** □ Muscular dystrophy, pseudohypertrophic—treatment with hexahydrocoenzyme $Q_4$, evaluation □ Hexahydrocoenzyme $Q_4$—evaluation of use in pseudohypertrophic muscular dystrophy □ Coenzyme $Q_4$ homologs—evaluated in muscular dystrophy treatment

Coenzyme $Q_{10}$, a relatively new vitamin (1), is widely distributed in mammalian species. Certain rodent tissue such as that of mice and rats may contain mostly coenzyme $Q_9$ but also some $Q_{10}$. The normal members of the coenzyme Q group, represented by I, differ in the number, *n*, of the isoprenoid units in the side chain. From the viewpoint of mammalian metabolism, coenzymes $Q_9$ and $Q_{10}$ may be regarded in the category of the oil-soluble group of vitamins such as vitamin A and vitamin D.



I

$n$ = 1–10

$n$ = 10 for human tissue

$n$ = $-CH_2CH{=}C(CH_3){-}CH_2(CH_2{-}CH_2{-}CH(CH_3){-}CH_2)_3H$ for hexahydrocoenzyme $Q_4$

Coenzyme $Q_{10}$ is naturally present in the human body. It was found in every organ and tissue analyzed (2) and, presumably, is in every cell of the human body that has mitochondria. Coenzyme $Q_{10}$ is a component of the bioenergetic reactions of respiration and coupled oxidative phosphorylation which reside in the inner mitochondrial membrane. The presence of coenzyme $Q_{10}$ in these electron-transfer processes is indispensable, and the molecule has the general structural specificities of a vitamin. It is evident that increasing deficiencies of coenzyme $Q_{10}$ would be increasingly deleterious to health and be reflected by some nature of disease, depending upon the distribution of the deficiency in the body.

Human muscle tissue and heart tissue obtained at autopsy from three individuals showed 20–30 mcg. $CoQ_{10}$/gram of wet weight (gww) tissue and 50–80 mcg. $CoQ_{10}$, respectively (2).

The response of mice with a hereditary muscular dystrophy to therapy with hexahydrocoenzyme $Q_4$ was first reported by Farley *et al.* (3), who observed that a dosage of 100 mg./kg. i.p. 7 days per week resulted in physical improvement but no increase of survival. Subsequently, Scholler *et al.* (4) increased the dosage to 250 mg./kg. 7 days a week by the oral route. At this higher level, there was a physical improvement in the dystrophic mice and the number of days from onset of therapy to death was four times that of the corresponding period for the control mice.

Zuckerman *et al.* (5) confirmed the response of these dystrophic mice to therapy with hexahydrocoenzyme $Q_4$ and found that the therapy increased by eightfold the "hanging-time" of the mice.

Analysis of the pooled livers, hearts, and kidneys of severely dystrophic mice showed a level of 112 mcg. of CoQ/gww. The corresponding level for the three pooled organs of nondystrophic littermates as controls showed 209 mcg. of CoQ/gww according to Nilsson *et al.* (6). Not only was there about a 50% deficiency of CoQ in these organs of the severely dystrophic mice, but the rate of biosynthesis of CoQ for severely diseased animals was about 50% lower than that of potentially dystrophic animals at a weaning age.

The muscular dystrophy (gene symbol dy), which occurred as a spontaneous autosomal mutation in a strain of mice 129 Re, causes muscular weakness, atrophy, and a reduced lifespan. These dystrophic mice show clinical, histological, and physiological similarities to myotonic dystrophy of man, Erb's dystrophy, and, except for the difference in inheritance, the Duchenne dystrophy (7). While there are several similarities between the genetic dystrophy of the mouse and the Duchenne type of muscular dystrophy in man, some metabolic differences also have been reported (8). Nevertheless, a trial of hexohydracoenzyme $Q_4$ in human muscular dystrophy was undertaken.

## MATERIALS AND METHODS

Nineteen boys, 5–17 years of age and in the early, intermediate, or late stages of pseudohypertrophic (*i.e.*, Duchenne type) muscular dystrophy, received synthetic hexahydrocoenzyme $Q_4$ *per os* for 1 year. The initial dosage of 250 mg./day was continued for 8 months and then increased to 1000 mg./day for an additional 4 months.

Prior to and during therapy, the observations cited in the *Results* section were made at intervals of from 1 to 12 months.

## RESULTS

The medication did not produce any untoward effects. All but one of the children increased in body weight during the year of therapy. The gains ranged from 3 to 14 lb. with an average of 7.5 lb. Blood pressure, pulse rates, and electrocardiographic tracings remained within pretherapy limits.

**Motor Performance**—None of the patients reported an increase in muscle strength and motor performance, nor was any improvement observed by their families. Six of the families felt that their children lost ground during the year of observation. The performance of 110 muscle groups against resistance and gravity was evaluated in each patient by a physical therapist prior to and at the end of the treatment period without evidence of improvement. Serial recordings of 105 successive contractions of either one or both hands in 105 sec. on an ergometer (9) confirmed the lack of an increase in strength.

Measurements of the time necessary for the patient to rise from the floor to a standing position did not indicate improvement in strength. The same conclusion was reached with respect to maximal extension of the lower leg while sitting on the edge of the examining table. When present, contractures at elbows, hips, and knees did not diminish during $CoQ_4$ therapy.

**Serum Enzymes and Solutes**—Serum creatine phosphokinase levels remained elevated in the high range characteristic of Duchenne muscular dystrophy (10, 11). The mean pretherapy value, based on three or more measurements in each child, was 473 ± 212 I.U. The 12th-month levels, comparable to those recorded after 3, 6, and 9 months of therapy, averaged out to 431 ± 212 I.U. The difference is not statistically significant by the *t*-test (12). Serum aldolase (13), when elevated, did not decrease, and alkaline and acid phosphatase (14) levels also remained within the pretherapy range.

Serum creatine and creatinine (15) did not change significantly during the 12 months of treatment and observation. Other serum solutes, comprising $CO_2$, Cl, Na, K, total protein, albumin, globulin, calcium, inorganic phosphorus (16, 17), uric acid (18), cholesterol (19), triglycerides (20), and nonesterified fatty acids (21), fluctuated within the normal range.

**Urine Data**—Urine creatine and creatinine excretion (15) and the renal clearance of the two measured during the 12th month of therapy were found to be within the pretreatment range. Similarly, the 24-hr. excretion of 17-ketosteroids (22), Porter–Silber chromogens (23), 11-desoxycortisol metabolites (24), and urinary pressors (aortic strip assay) (25) was of the same order of magnitude in the control and treatment periods.

**Glucose Tolerance and Serum Insulin**—Oral glucose tolerance, judging by the Glucose Tolerance Sum (26), based on the 0-, 0.5-, 1-, and 2-hr. blood glucose levels following 1.75 g. of glucose/kg. of body weight, remained below 500. As established in evaluations in this laboratory (26), each of these glucose tolerance tests would be classified as normal by the World Health Organization, the U. S. Public Health Service, the British Diabetic Association, and Fajans and Conn. Also, the serum immunoassayable insulin levels (27) recorded in the glucose tolerance test performed in the 12th month of treatment were about the same as those observed in the pretherapy period; the difference between the mean values was not statistically significant.

Serum growth hormone (27) and inorganic phosphorus (17) levels recorded in these glucose tolerance tests were not affected by the 1 year of treatment.

**Other Indexes**—The relative blood cell volume, the hemoglobin level, and the sedimentation rate did not change during treatment. This was also true of hepatic indexes including serum bilirubin, cephalin flocculation, and thymol turbidity and the thyroidal indexes [serum PBI (28), uptake of exogenous $T_3$ by blood cells *in vitro* (29), and the thyroidal $^{131}I$ uptake at 24 hr.].

## DISCUSSION

In part, this trial of hexahydrocoenzyme $Q_4$ in human pseudohypertrophic muscular dystrophy was undertaken to follow up the demonstration that this compound improved hind-limb strength in genetically dystrophic mice and prolonged their survival (4). Coenzyme Q is functional at two enzyme sites in the mitochondrial electron-transfer processes, one in succin-oxidase and the other in DPNH-oxidase.

As a working hypothesis, Farley *et al.* (3) and Gale *et al.* (2) suggested that the muscular dystrophy occurring as a genetic trait in mice or in humans might result from a deficiency of coenzyme Q which might result primarily from a genetic defect in its biosynthesis. The deficiency might be augmented by absence of dietary factors required for the biosynthesis and peroxidation of coenzyme Q and its isoprenoid precursors. A deficiency of coenzyme Q would lead to an impairment of electron transfer in the enzyme systems of succin-oxidase and DPNH-oxidase and, in turn, there could be a deficiency in the biosynthesis of adenosine triphosphate through the coupled oxidative phosphorylation which would result in the low levels of adenosine triphosphate that characterize dystrophic muscle (30).

These trials with hexahydrocoenzyme $Q_4$, undertaken as a possible test of this hypothesis, did not result in improved muscle strength and motor performance in this group of patients with pseudohypertrophic muscular dystrophy of the Duchenne type. Apparent improvement in hand strength during the early months of treatment was not continued and probably reflected increased skill

in manipulating the rubber bulb. Theoretically, the medication could have retarded a loss of muscle strength without evident improvement, but the control observations of the natural history of this disorder (31) exclude this possibility. Moreover, in these studies the ingestion of hexahydrocoenzyme $Q_4$ at a dosage of 250–1000 mg./day during a 12-month interval was not associated with any decrease in serum creatine phosphokinase nor with statistically significant changes in serum or urine creatine and creatinine; other blood and serum solutes; urinary steroids; hematologic, hepatic, or thyroidal indexes; or oral glucose tolerance.

When the dystrophic mice were given hexahydrocoenzyme $Q_4$ at a dosage of 10 mg./kg. p.o. 5 days per week, the improvement in physical performance was marginal; but at 100 mg./kg. i.p. 7 days per week, improvement of physical status was clearly evident (3). At 250 mg./kg. p.o. 7 days per week, not only was physical performance improved, but survival was significantly increased (4). In comparison, these 19 boys, 5–17 years of age and in early, intermediate, or late stages of Duchenne dystrophy, received approximately 5 mg./kg. of hexahydrocoenzyme $Q_4$ for 8 months and then approximately 20 mg./kg. for an additional 4 months based on an average body weight of 50 kg. Based on a direct extrapolation of the most effective dosage in the dystrophic mice, these boys received only about one-fiftieth of the extrapolated dosage for 8 months and only about one-tenth of the extrapolated dosage for the remaining 4 months. On the other hand, the dosage administered to the boys can seem high when one considers that the total body content of coenzyme $Q_{10}$ appears to be in the range of 0.5–1.5 g. (2). Assuming a deficiency of coenzyme $Q_{10}$ in the body of a boy with Duchenne dystrophy, his total body content of coenzyme $Q_{10}$ could be expected to be not much less than perhaps 75% of 0.5–1.5 g. While the actual clinical dosage might seem adequate to restore such a deficiency of coenzyme $Q_{10}$, this reasoning may be inappropriate on the basis of a comparison of the effective dosage for the dystrophic mouse with this animal's total body content of coenzyme Q. The effective dosage for the mouse is far in excess of the total body content of coenzyme Q. The dosage for the mice and the clinical dosage may correlate with a report by Rosenberg (32), who summarized the background on "inherited" vitamin-dependency states which have been defined as genetic disturbances that lead to specific biochemical abnormalities which involve catalysis by a vitamin, but respond only to "massive" amounts of the vitamin. "Massive" amounts may be from 10- up to 1000-fold the dose level of the same vitamin which corrects a primary deficiency state due to dietary inadequacy. Reaction specificity, genetic etiology, and quantitative responsiveness are understood to characterize a genetic vitamin-dependency state. A primary vitamin-deficiency state is generally acquired through dietary inadequacy and is generally corrected by very low dose levels of the vitamin. On such a genetic basis, the effective dosage of hexahydrocoenzyme $Q_4$ for the dystrophic mice might be explained, and the dosage range of 250–1000 mg./kg. for these boys may have been low. However, the absence of benefit from the clinical dosage may be due to the intrinsic inactivity of hexahydrocoenzyme $Q_4$ in the Duchenne type of dystrophy.

Despite the absence of any beneficial or any other effects of hexahydrocoenzyme $Q_4$ in these studies, the possibility still exists that higher homologs might stay the course of muscular dystrophy. It recently became known that these mice with a hereditary dystrophy also respond to treatment with coenzyme $Q_7$ (33); companion studies (34) revealed a deficiency of coenzyme Q in the succinate dehydrogenase–coenzyme Q reductase of the hearts and hindleg muscles of the dystrophic mice and that this deficiency increases with increasing severity of the dystrophy. Studies with the higher homologs of coenzyme Q are certainly in order. It is the highest homolog, *i.e.*, coenzyme $Q_{10}$, that is biosynthesized in human tissues and that ultimately will be the coenzyme Q of choice for final appraisal. These studies were initiated with hexahydrocoenzyme $Q_4$ because other homologs were not available in quantities sufficient for clinical trial.

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