RESPONSE OF GENETICALLY DYSTROPHIC MICE TO THERAPY WITH HEXAHYDROCOENZYME Q_4*

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Received June 26, 1966

Mice having genetic dystrophy have been treated therapeutically with hexahydrocoenzyme Q_4 and there was distinct improvement, even to the extent that severely dystrophic animals which were also in poor health, responded and were able to walk using all their legs. These results are viewed as a progress report. Perhaps though, these results forecast a vitamin-like role for coenzyme Q in the treatment of muscle disease of man, including muscular dystrophy.

The first vitamin-like activity for hexahydrocoenzyme Q_4 was demonstrated *in vivo* by both prophylactic and curative activity in the dystrophic rabbit produced by nutritional design (Wagner *et al.*, 1964; Smith *et al.*, 1965; Smith *et al.*, 1966). Next, the vitamin-like activity was revealed for the anemic and dystrophic rhesus monkey (Fitch *et al.*, 1965), and this activity in the primate has been confirmed and extended (Farley *et al.*, 1966).

Such nutritional dystrophy in both the rabbit and the monkey was originally described as a "vitamin E-deficiency," but today new consideration is being given to these nutritional dystrophies as "antioxidant-deficiencies" (Dam, 1957; Horwitt, 1965); on this basis, the very effective activity of vitamin E in preventing and curing this nutritional dystrophy may be considered as that of a nonspecific chemical antioxidant. Some synthetic antioxidants of totally unrelated structure to vitamin E exhibit the same protection (Crider *et al.*, 1961).

It has been evident for years that this nutritional dystrophy, whatever may be its cause, is clearly metabolically different from genetic dystrophy in mam-

*Coenzyme Q. LXXVI.

malian species and particularly in man. Supporting this differentiation is the knowledge that vitamin E is ineffective therapeutically in the treatment of the genetic dystrophy of mice (Tubis et al., 1959) and muscular dystrophy in man (Dreyfus and Shapiro, 1962).

Genetic dystrophy in mice has been known for some time and studied extensively from the hereditary point of view (Staats, 1965). No effective long-term therapeutic response of basic biochemical significance has yet been reported for genetic dystrophy in such mice although, indeed, a variety of treatments have been explored. For example, massive vitamin therapy has been explored unsuccessfully (Tubis et al., 1959). Certain anabolic steroids produced a longevity in lifespan but no alteration in the disease syndrome (Dowben, 1959; Borgman, 1963). Neostigmine has been reported to produce a temporary improvement in muscle strength and dystrophic state (Baker et al., 1959).

Two statements of basic knowledge which led us to open this study of treatment with CoQ of genetic dystrophy in mice are: (1) CoQ is an indispensable component having some structural specificity in the electron transfer process of respiration and coupled biosynthesis of ATP, as evidenced by its activity in the succinoxidase and DPNH-oxidase systems (Folkers et al., 1966), and in comparison, vitamin E is inactive in both enzyme systems; (2) CoQ has exhibited vitamin-like activity in the nutritional dystrophy of rabbits (Smith et al., 1966) and the primate (Fitch et al., 1965).

Genetically dystrophic male mice, Jax/129 (dydy), were obtained from the Jackson Laboratory, Bar Harbor, Maine, and treated with hexahydrocoenzyme Q_4. During therapy they were maintained on standard maintenance pellets. These mice require considerable care and attention to maintain them in a reasonably good state of health. The data from two separate experiments are presented in Table I.

Of the eight control animals, the dystrophic status of seven mice progressively deteriorated. The scoring of the control animals revealed a general increase in the dystrophy from mild to severe during a period comparable with periods of observation for the treated animals. Only one of the control animals

showed no change in its dystrophic status.

Of the dystrophic animals treated with hexahydrocoenzyme Q_4, all ten mice showed clear physical improvement and reduction of dystrophy. Four of the ten treated control mice were severely dystrophic and in poor health at the initiation of therapy, but nevertheless responded to therapy and were able to walk using all legs, particularly the hind quarters.

An interpretation of the apparent vitamin-like activity of this CoQ_4 in the genetic dystrophy of mice may be stated as follows. Coenzyme Q_{10} is most commonly found in mammalian tissue although mice frequently reveal the presence of coenzyme Q_9. Coenzyme Q_{10} and Q_9 are biosynthesized within the mammalian body. Further, the actual biosynthetic sequence of coenzyme Q from p-hydroxybenzoic acid (HBA) has been largely elucidated (Olsen et al., 1965, 1966). This biosynthetic sequence is complex and requires numerous proteins, lipids, vitamins, inorganic ions, and other cofactors. Such a complex biosynthesis appears to offer many metabolic possibilities for genetic "blocks." From such inborn errors of metabolism, an inadequacy of functional levels of coenzyme Q could result with concomitant impairment of electron transfer. If a deficiency of CoQ resulted from such a metabolic "block," then therapeutic administration of CoQ might well restore functional levels for electron transfer and corresponding health. Since hexahydrocoenzyme Q_4 is fully active (Folkers et al., 1966) in the succinoxidase system as compared to coenzyme Q_{10}, and since this CoQ_4 is therapeutically active in nutritional dystrophy, it is evident that the response of these mice with genetic dystrophy to therapy with this CoQ_4 could signify that the dystrophy of these mice is possibly caused by a genetic "block" in the biosynthesis of CoQ.

The failure of vitamin E to exhibit therapeutic activity in the genetic dystrophy of mice and humans would be expected, since vitamin E should have no effect upon the mechanism of the genetic "block" itself and since it can not substitute for CoQ in electron transfer.

The extension of these favorable data to the therapy of dystrophy of

hereditary origin in other experimental species and particularly to the muscular dystrophies in humans is now very attractive.

Acknowledgments. - We wish to thank Mrs. Anne H. Tait and Miss Winifred M. Ball for skillful and dedicated assistance, and the Muscular Dystrophy Associations of America, Inc., F. Hoffmann-La Roche and Co., Ltd., Basel, Switzerland, and Merck and Co., Inc., Rahway, New Jersey, for their support.

Table I - RESPONSE OF GENETIC DYSTROPHY TO HEXAHYDROCOENZYME Q_4

Dose	Status at Day 0	Days of Therapy	Evaluation of Dystrophy Status/Days
Control	1+	-	4+/63 (a)
	1+	-	4+/63 (b)
	1+	-	1+/35
	1+	-	3+/35
	1+	-	3+/35
	1+ - 2+	-	4+/72 (c)
	1+ - 2+	-	3+/49 (c)
	1+	-	3+/49 (c)
10 mg/kg/PO, 5 days/week	1+	31	0/32 (d)
	1+	31	0/32 (d)
	1+	31	0/32 (d)
	4+ (e)	15	1+/87
	3+ (f)	15	0/64
	3+ (f)	15	1+/64
100 mg/kg/IP, 7 days/week	2+	49	0/35-42
	4+	25	0/18
	2+	66 (g)	0/37 (h)
	2+	34	0/22

Definition of Status

0 Able to walk normally.
1+ Able to use front, but only one hind leg.
2+ Able to use front, but not hind legs.
3+ Increased dystrophy; hind legs very distended.
4+ Marked dystrophy and poor physical appearance.

(a) Died/71.
(b) Alive/98.
(c) Control animals which were subsequently treated.
(d) Also 0/76; 1+/98.
(e) Treatment begun day 72 on control.
(f) Treatment begun day 49 on control.
(g) Dose level reduced to 10 mg/kg/day IP, 7 days/week on day 22, and to 5 days/week on day 29.
(h) Status 0-1+/66.

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