Effects of Hexahydrocoenzyme Q_4 on Murine Muscular Dystrophy* (33426)

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The administration of hexahydrocoenzyme Q_4 to a mutant strain of mice afflicted with an hereditary disease bearing a resemblance to human muscular dystrophy has been reported to result in a distinct improvement in muscle strength (1) as judged by gross observations of the ability of the mice to walk. Hexahydrocoenzyme Q_4 also has been reported to reverse the nutritional muscular dystrophy of rabbits (2, 3). This study was undertaken to quantify changes in muscle strength and in the electrolyte composition of residual muscle which might follow the administration of hexahydrocoenzyme Q_4 to dystrophic mice. Concerning the latter point, muscle from dystrophic mice contains less potassium and more sodium and chloride than muscle from normal mice (4, 5). Dystrophic muscle also has an abnormally high potassium efflux rate (6). Return of the abnormal electrolytes in dystrophic muscle toward normal might be expected to accompany a reversal of the dystrophic process.

Materials and Methods. Strain 129/Re mice and dystrophic mutants of this strain were obtained as weanlings from the Jackson Memorial Laboratory, Bar Harbor, Maine. The details of handling and feeding were described previously (5). Beginning on the fifty-fourth day of age, 6 dystrophic and 6 parent strain mice were given hexahydrocoenzyme Q_4 (20 μg/g of body weight, i.p.) as an emulsion in water containing 0.05% Tween-80. The hexahydrocoenzyme Q_4 was a generous gift of Dr. George E. Boxer, Merck Institute for Therapeutic Research. Equal numbers of dystrophic and parent strain control animals were given vehicle alone. Muscle strength was assessed by measuring the length of time the animals could hang on a vertical screen(s). After 4 weeks of treatment, the animals were sacrificed and a dry, fat-free muscle powder (DFFM) was prepared from the rump muscles. Sodium and potassium were determined in a 0.6 *N* perchloric acid extract of DFFM using a Technitron flame photometer. Chloride was determined in a 0.75 *N* nitric acid extract of DFFM by the method of Cotlove *et al.* (7). Potassium efflux from excised *peroneus longus* muscle was measured using ^{42}K by a modification (5) of Zierler's procedure (6) in a temperature controlled system at 26°.

Results and Discussion. The dystrophic mice treated with hexahydrocoenzyme Q_4 were generally more active, had a smoother coat, and appeared more healthy than the untreated dystrophic animals. There was no significant difference in weight between control and treated dystrophic animals. The control dystrophic mice were able to hang on a vertical screen an average of 5.5 sec during the entire course of observation. While the treated animals showed little difference from the controls during the first week of treatment, the hanging time then increased rapidly to an average of 44.5 sec at the end of 4 weeks (Fig. 1). One treated dystrophic mouse could hang in excess of 5 min at the end of the treatment period. All parent strain animals, treated or control, could hang longer than 5 min at all times.

The analyses of the electrolyte content of residual muscle are summarized in Table I. The lower potassium and higher sodium and chloride content of dystrophic muscle compared to normal muscle reported previously (4, 5) was found again in these studies. There was no significant difference, however, in the electrolyte content of muscle from treated dystrophic mice or treated parent strain mice when compared to muscle from the corresponding controls. The rates of potassium efflux were greater from muscle of dystrophic animals than from muscle of parent strain ani-

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TABLE I. Skeletal Muscle Electrolytes and ^{42}K Efflux Rates in Dystrophic and Parent Strain Mice Treated with Hexahydrocoenzyme Q_4.

	Dystrophic		Parent strain	
	Treated	Control	Treated	Control
Muscle: Na (meq/kg of DFFM)	167 ± 16	171 ± 11	124 ± 24	109 ± 6
K (meq/kg of DFFM)	381 ± 27	378 ± 23	387 ± 30	418 ± 27
Cl (meq/kg of DFFM)	102 ± 21	108 ± 17	79 ± 13	61 ± 5
H_2O (%)	68.7 ± 1.5	69.4 ± 1.8	72.8 ± 0.6	72.2 ± 0.6
^{42}K efflux from excised *peroneus longus* muscle (hr^{-1} ± SEM)	0.679 ± 0.052	0.763 ± 0.045	0.337 ± 0.020	0.309 ± 0.012

mals (whether or not they had been treated). While the rates of potassium efflux were lower from muscle of both groups of treated animals than from the corresponding untreated controls, *t* tests showed that $P > 0.05$ in both instances. Treatment did not result in the potassium efflux rate of dystrophic muscle becoming at all comparable to that of normal muscle.

Coenzyme Q appears to be an intermediate carrier in the electron transport chain associated with oxidative phosphorylation in mitochondria (8). Modest elevations of the succinate dehydrogenase and cytochrome oxidase levels have been found in muscle from dystrophic mice (9, 10). On the other hand, no significant differences in respiration were found in isolated mitochondria from dystrophic muscle and normal muscle (11).

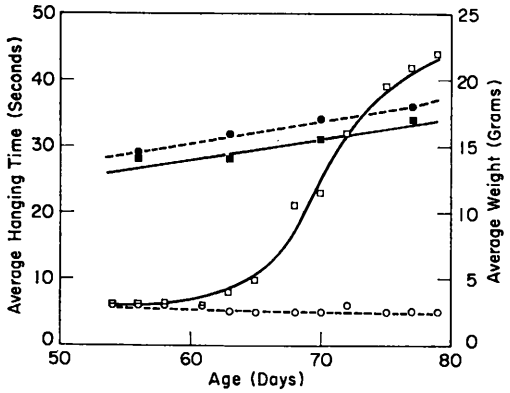

FIG. 1. Six dystrophic mutants of 129/Re mice were treated with hexahydrocoenzyme Q_4 (20 μg/g of body wt.). The average hanging time on a vertical screen, (□); and average wt. (■); are compared with those for untreated dystrophic mice, (○), and (●), respectively.

Coenzyme Q is a constituent of many types of membranes (12), and it has been shown to stabilize erythrocytes against hemolysis by hydrogen peroxide (13). Leakiness of the plasma membrane has been thought to be a defect in dystrophic muscle (4–6). The abnormally high potassium efflux from dystrophic muscle did not revert toward normal in animals treated with hexahydrocoenzyme Q_4, nor were there significant changes in the abnormal electrolyte composition of dystrophic muscle. It is difficult, therefore, to ascribe the salutary action of hexahydrocoenzyme Q_4 to an effect upon cation permeability. While the mechanism of action of hexahydrocoenzyme Q_4 remains unclear, it produces an undoubted increase in the muscle strength of dystrophic mice. Further studies will undoubtedly clarify its mechanism of action and may shed light on the dystrophic process itself.

Summary. The administration of hexahydrocoenzyme Q_4 (20 μg/g of body weight) resulted in an increase in muscle strength of dystrophic mice with a hanging time on a vertical screen of 44.5 sec after 4 weeks of treatment compared to 5.5 sec for untreated controls. A slight decrease in the efflux rate of potassium accompanied treatment, but there was no change in the electrolyte composition of dystrophic muscle.

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Influence of Chloramphenicol and Cetophenicol on Antibody Formation in Mice (33427)

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Chloramphenicol has been shown to inhibit both the specific primary antibody response and priming for a subsequent secondary response, as well as to inhibit induced protein synthesis in mammalian cell-free systems (1–4). The influence of chloramphenicol on various expressions of the immune response and on mammalian cell function has recently been reviewed (5). Cetophenicol, an antibiotic analog of chloramphenicol in which the nitro group in the para position of chloramphenicol is replaced by an acetyl group (6), has an antibacterial spectrum identical with that of chloramphenicol (7). Cetophenicol, like chloramphenicol, has been shown to inhibit protein synthesis *in vitro* (4). In this initial report the inhibition of antibody formation by chloramphenicol is demonstrated at substantially lower than usually required doses of antibiotic, made possible by use of the technique of localized hemolysis in agar (8). Further, the two antibiotic analogs are shown to differ qualitatively in their opposite effects upon the increase in numbers of hemolysin-forming spleen cells induced by bacterial endotoxin (9, 10). Chloramphenicol suppresses and cetophenicol enhances this elevation in numbers of antibody-forming cells, whereas both antibiotics suppress the specific primary response to foreign erythrocytes. Studies on priming for a subsequent secondary response to specific antigen, to be reported separately, reveal the same distinction between the two analogs: enhancement, instead of suppression, of priming by cetophenicol despite inhibition of the primary response equivalent to that produced by chloramphenicol.

Materials and Methods. Female CD-1 mice (Charles River), weighing 20–22 g, were injected with sheep red blood cells, i.v., at a dose of 10^8 cells, or with *Salmonella abortus equi* Boivin endotoxin (Difco), i.p., at a dose of 10μg. Chloramphenicol (D-threo-2-[2, 2-dichloroacetamido]-1-[*p*-nitrophenyl]-1,3-propanediol) or cetophenicol (D-threo-1-[*p*-acetylphenyl]-2-[2, 2-dichloroacetamido]-1, 3-propanediol) was administered, i.p., at doses of 1 or 2 mg, 3 times/day (approx 150 or 300 mg/kg/day). Treatment with antibiotic was given 6 and 2 hr before and 2 hr after injection of red cells or endotoxin, and was repeated the next day at 4-hr intervals. The following day, 42 hr after immunization or endotoxin treatment, spleens were harvested for assay. Numbers of hemolysin-forming spleen cells were determined by the technique of localized hemolysis in agar (8) using sheep red blood cells and 1/5 of the cell suspension of the whole spleen, as previously described