Coenzyme Q. LX. Vitamin-Like Activity of Coenzyme Q.* (29969)

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Coenzyme Q (ubiquinone) has vitamin-like activity in laboratory animals under certain experimental conditions. This vitamin-like activity was first demonstrated for a chromanol of the coenzyme Q group; however, a chromanol has not yet been identified as a naturally occurring form of coenzyme Q. Vitamin-like activity for the naturally occurring quinone forms of coenzyme Q was first demonstrated in the dystrophic rabbit (1,2,3). The data on the vitamin-like activity of the quinone forms have been extended.

Hexahydrocoenzyme Q_4 cured rabbits of the nutritionally induced dystrophy, and prevented appearance of the dystrophy by prophylactic therapy; this new finding is equivalent to a vitamin-like activity *in vivo* for coenzyme Q. This nutritional dystrophy in the rabbit is the syndrome which has been known for years to respond to vit. E. In the anemic and dystrophic rhesus monkey, coenzyme Q_{10} therapy caused a reticulocytosis, and a complete hematologic remission occurred after therapy with a compound of lower molecular weight, hexahydrocoenzyme Q_4(4). It is apparent that the quinone members of CoQ deserve new emphasis since they are the compounds of the group which have been identified to date in nature.

It is evident that the biological activities, *in vivo,* of the 6-chromanol of hexahydrocoenzyme Q_4 and vit E are very similar in the rat(5,6), chicken(6), rooster sperm(7,8), rabbit(6), monkey(9), and man(10,11). Although the similarity exists, it is becoming increasingly apparent that there are biochemical differences in the responses to coenzyme Q and vit E(12,13). This current study is also concerned with further elucidation of such differences.

Methods. Nutritional muscular dystrophy was produced in New Zealand white rabbits of mixed sex essentially as described(1,6). The vit E-deficient diet(14) consisted of sucrose, starch, vitamin-free test casein;† cod liver oil and stripped lard† were the lipids. Under these conditions, animals weighing from 0.6 to 1.1 kg would become dystrophic within 4 to 6 weeks. The progression of the muscular dystrophy was measured by loss of the righting reflex and other physical responses, such as incoordination and loss of weight, and the ratio of excretion of creatine to creatinine. A positive physical criterion to guide therapy of the muscular dystrophy was when the animal could no longer right itself after being placed on its right side 20 times. The rabbit usually exhibited severe incoordination of either or both front and hind extremities. Weight losses usually preceded the appearance of the other physical signs.

Creatine and creatinine excretion was determined(15) on periodic 24-hour urine samples.

The compounds were administered, on failure of righting, after 20 falls(1,6) were observed (unless otherwise noted), either intravenously in a 5% dimethylacetamide and 2.5% Emulfor EL 620 in pyrogen-free water solution, or orally dissolved in stripped corn oil.† Administration of compounds for prophylactic experiments was started the same day the animals were placed on the experimental diet.

The therapeutic response of the treated animals was determined by the return of the righting reflex and loss of the incoordination and a normalization of the creatine-creatinine excretion. Both of these physical and biochemical responses were required for declaration of activity for a test substance. If only one response was observed, a $\pm$ response was recorded, and if neither response was observed, the result was considered negative. These criteria for judging responses of the animals to compounds, administered at the same dose level, do not permit precise quantitative measurement of activities, but

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† General Biochemicals, Chagrin Falls, Ohio.

TABLE I. Administration of Coenzyme Q_{10} to Rabbits Having Nutritional Muscular Dystrophy.

	No. of animals	Avg day signs observed	Avg days of survival after appearance of signs
None	6	28	5
Coenzyme Q_{10}—40 mg/day orally	2	37	5
Coenzyme Q_{10}—80 mg/day orally	2	30	5
Avg of all experimental animals	60	33	—

do serve to assign relative activities of the compounds.

The regaining of weight by the animals does not necessarily parallel the increase in physical capacity and the biochemical response, but usually a regain of weight or at least a slowing of the rate of losses accompanies the other criteria of therapeutic response.

Results. Control rabbits. In each experiment, positive and negative control animals were used. Two or more rabbits out of each group of 24 animals were either not treated or were treated with the vehicle alone; there was no difference in the behavior of these two types of controls. Table I shows results obtained for 6 such control animals. The control animals developed physical signs after an average of 28 days; the over-all average of all animals was 33 days. The negative control animals survived for an average of 5 days after the appearance of severe physical signs of dystrophy. None of the controls showed spontaneous recovery; however, in some rare instances, an animal lingered for many days.

Positive control animals were treated both prophylactically and curatively. Prophylactic controls were given 10 mg of α-tocopherol orally 3 times a week. This amount of α-tocopherol was more than sufficient to prevent the appearance of either physical signs of dystrophy or an increase of creatine excretion during the experimental period. Three to four weeks were required for the animals to develop signs of dystrophy after the α-tocopherol was removed. On a curative basis, an animal which had shown physical signs of dystrophy by failure of righting was injected intravenously with a single dose of 20 mg of α-tocopherol. After about 3 days, response was noted with a return of the righting reflex and the normalization of the creatine excretion. Seven to ten days were required for dystrophic signs to reappear and for the creatine excretion to be elevated again, after the single dose of α-tocopherol was administered.

Hexahydrocoenzyme Q_4. The results of treating 7 rabbits with hexahydrocoenzyme Q_4 at a level of 20 mg/kg/day are shown in Table II. For each rabbit, the creatine-creatinine ratio was decreased below 1.0 and usually well below. In 2 rabbits, the animal did not exhibit physical response to the treatment at this dose level and died. In general, recovery was noted after 5-8 days of treatment. Weight was not always recovered as when the animals were treated with a single dose of α-tocopherol, but full weight responses were noted in 5 of the 7 animals treated, and a partial weight response was noted in the other 2 animals.

Coenzyme Q_{10}. The results of prophylactic administration of coenzyme Q_{10} are shown in Table I. Physical signs were observed on the 37th and 30th day for the animals receiving 40 and 80 mg/day orally, respectively. In the average of 6 negative control animals signs were observed on the 28th day, and in the average for all animals used in curative-type experiments signs appeared by the 33rd day. There was no significant beneficial effect in prophylactic administration of coenzyme Q_{10} in these tests.

2,3,5-Trimethyl-6-phytylbenzoquinone. Data obtained by treating 9 rabbits with trimethylphytylbenzoquinone are summarized in Table II. All of the animals except 2 were treated after the onset of signs. These 2 animals were treated after having been on the deficient diet for 28 days. By this time, creatine excretion was elevated, but the animals had not yet developed physical signs. In neither case was there observed any decrease in creatine excretion or change in time of onset of the physical signs.

One animal responded to the quinone of vit E. Complete response of both the normalization of the creatine excretion and recovery of the righting reflex was observed in

TABLE II. Biological Activity of Quinones in Rabbits Having Nutritional Muscular Dystrophy.

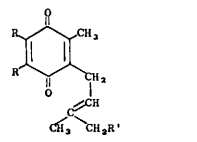
	Weight (kg)	Total mg inj (20 mg/kg/day)	Creatine/ Creatinine	Mortality	Response*
Hexahydrocoenzyme Q_4	1.0	140 (5)†	<1.0	Survived	+
	.9	90 (5)	"	"	+
	1.2	160 (8)	"	Died	±
	1.1	140 (7)	"	Survived	+
	1.4	120 (6)	"	"	+
	1.3	200 (9)	"	"	+
	1.0	100 (5)	"	Died	±
	Summary				5 +, 2 ±
2,3,5-Trimethyl-6-phytyl-benzoquinone	1.1	120 (6)	>2.0	Died	—
	1.0	83 (5)	"	"	—
	1.0	100 (5)	"	"	—
	1.4	135 (6)	"	Survived‡	—
	1.0	127 (7)	"	Died	—
	.7	125 (7)	"	Survived‡	—
	1.3	340 (17)	<1.0	Survived	+
	1.0	120 (6)	>2.0	Died	—
	.8	40 (2)	"	"	—
	Summary				8 —, 1 +

* +, Activity if both the creatine and physical signs were normalized; ±, if only response to one sign was observed; —, if no response to both criteria was observed.

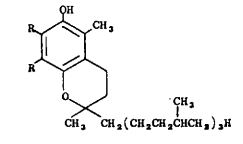
† Days.

‡ Treated before appearance of signs.

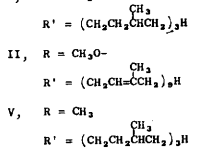
about 5 days after beginning the treatment. Injections were continued for a total of 17 days to see if dystrophic signs would reappear during treatment, but none were seen. After the treatment was discontinued, the animal again developed dystrophic signs. This time, it did not respond to treatment with trimethylphytylbenzoquinone. Eight of the nine animals treated with trimethylphytylbenzoquinone did not respond in any way; one did respond.



I, R = CH_3O-
R' = $(CH_2CH_2\overset{CH_3}{\overset{|}{C}}HCH_2)_3H$

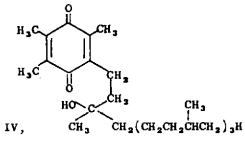


III, R = CH_3O- ; IV, R = CH_3-



II, R = CH_3O-
R' = $(CH_2CH{=}\overset{CH_3}{\overset{|}{C}}CH_2)_9H$

V, R = CH_3
R' = $(CH_2CH_2\overset{CH_3}{\overset{|}{C}}HCH_2)_3H$



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Discussion. Hexahydrocoenzyme Q_4 (I) has vitamin-like activity which was exhibited when this quinone was administered to the dystrophic rabbit. The activity was evidenced by normalization of the creatine excretion, and by general physical recovery. The variations in the individual responses of the rabbits are believed to be due to the nature of the experimentation; the inherent vitamin-like activity of the quinone is clearly observed. These data extend and confirm the previous finding(1-3) that hexahydrocoenzyme Q_4 is active, both curatively and prophylactically, for the nutritional dystrophy in the rabbit.

Hexahydrocoenzyme Q_4 does not occur naturally in the tissue of the rabbit; it is a synthetic quinone closely analogous in structure to coenzyme Q_{10} (II) which is in the tissue of the rabbit(16). Hexahydrocoenzyme Q_4 has about one-half the molecular weight of coenzyme Q_{10} which is 862. It is well recognized that the unique CoQ_{10}, although naturally occurring widespread in mammalian tissue, is a substance which is difficult to disperse in *in vitro* systems and to administer to animals in such a manner that inherent biological reactivity can occur. Since it is now demonstrated that hexahydrocoenzyme Q_4 has vitamin-like activity in the dystrophic rabbit, it is reasonable to predict that coenzyme Q_{10} would exhibit the same activity in the dystrophic rabbit if adequate absorption and transport occurred after administration. The prophylactic administration of coenzyme Q_{10} at an equal and double molar dose level of that used for hexahydrocoenzyme Q_4 did not result in a significant therapeutic response

of the rabbit. This negative result in the rabbit is apparently due to failure of the administered CoQ_{10} to reach its mitochondrial and microsomal sites as demonstrated in the monkey. The response of the dystrophic rabbit to hexahydrocoenzyme Q_4 and the lack of a response to CoQ_{10} are analogous to the results of the administration of these same two quinones to the anemic and dystrophic rhesus monkey(4). Administration of CoQ_{10} to the deficient monkey did elicit a reticulocytosis, but otherwise the hematological response was incomplete; obviously, some of the administered compound reached its active sites. An inadequate dose level of hexahydrocoenzyme Q_4 in the deficient monkey likewise elicited only a reticulocytosis, but an increased dose of hexahydrocoenzyme Q_4 elicited a complete hematological response.

The CoQ_4-chromanol (III) and α-tocopherol (IV) are both 6-chromanols, and both elicit similar therapeutic responses in the dystrophic rabbit. As yet, no significant differentiation of the responses in the rabbit to CoQ_4-chromanol and α-tocopherol is evident. It is reasonable to believe, however, on the basis of the known mitochondrial biochemistry of CoQ that there may be some fundamental difference in the requirement of the rabbit for vit E and CoQ. In considering interpretations of sparing effects, it is not yet clear whether CoQ is sparing vit E or *vice versa,* or whether both CoQ and vit E are intrinsically required.

In the interest of fundamentals, it was desirable to compare quinone forms of both the CoQ and vit E groups in the dystrophic rabbit. 2,3,5-Trimethyl-6-phytylbenzoquinone (V) is related as a quinone to α-tocopherol as hexahydrocoenzyme Q_4 is related to its corresponding 6-chromanol; further, these respective 2 quinones and 2 chromanols are differentiated only by 2 methyl groups *vs* 2 methoxy groups. The isoprenoid side chain and all other structural features of the 2 pairs of compounds are identical. Hence, these 2 pairs of compounds are appropriately structurally related for meaningful comparison of their biological activities. 2,3,5-Trimethyl-6-phytylbenzoquinone should not be confused with the oxidation product of α-tocopherol which is α-tocopherylquinone (VI).

2,3,5-Trymethyl-6-phytylbenzoquinone exhibited very low activity in the dystrophic rabbit as compared with the activity of α-tocopherol and hexahydrocoenzyme Q_4. In only one of 9 rabbits treated with 2,3,5-trimethyl-6-phytylbenzoquinone was a significant response observed. For 2 quinones (I and V) of such similar structure, it is understandable, on the basis of classic structure-activity relationships, that 2,3,5-trimethyl-6-phytylbenzoquinone can have a low degree of activity as compared to that of hexahydrocoenzyme Q_4. A substance of low activity could give such variable response by gross criteria.

The purity of the 2,3,5-trimethyl-6-phytylbenzoquinone was examined to detect any contamination of undesirable α-tocopherol. The quinone contained less than 0.05% of α-tocopherol as judged by the absence of any easily oxidizable material when a thin layer chromatogram (silica gel G) of the newly synthesized sample was sprayed with Emmerie-Engel reagent.

2,3,5-Trimethyl-6-phytylbenzoquinone was previously found to be inactive for preventing the resorption-gestation syndrome in rats(17) and for preventing encephalomalacia in chicks (6). However, it has been reported that 2,3,5-trimethyl-6-phytylbenzoquinone(18) at a single dose level of 50 mg elicited a positive response in 4 dystrophic rabbits; this dosage is considerably less than that which we have used (Table II) and which gave very low activity.

Summary. Vitamin-like activity for hexahydrocoenzyme Q_4 in the dystrophic rabbit is demonstrated. This activity for a quinone member of the CoQ group is comparable to the activity of α-tocopherol and CoQ_4-chromanol which has previously been demonstrated. By contrast, a sample of 2,3,5-trimethyl-6-phytylbenzoquinone, which was free of contamination with α-tocopherol, exhibited only a low order of activity in the dystrophic rabbit. The dystrophic rabbit failed to recover when coenzyme Q_{10} was administered orally; it is apparent that the administered CoQ_{10} failed to reach its mitochondrial and microsomal sites since a lower homolog, hexahydrocoenzyme Q_4, exhibits activity in the dystrophic rabbit. It is considered that CoQ_{10}, which is normally present in rabbit tissue,

does inherently have this vitamin-like activity.

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The Dispensability of Complement for Antidevelopmental Action of Antisera Against Fertilizin in Sea Urchin Eggs.* (29970)

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Antisera that are prepared in rabbits against the purified fertilizin of sea urchin eggs have been previously shown(1-6) to have cytotoxic effects. When added to unfertilized eggs such antisera inhibit fertilization and, after prolonged treatment, cause visible blistering and cytolysis. When added to fertilized eggs, in various stages of development, the antisera can block cleavage abruptly, nuclear as well as cytoplasmic division being inhibited. Visible cytolysis follows some time later.

According to present evidence(5) fertilizin is a primary constituent of the plasma membrane of the egg and extends through the overlying vitelline membrane in the form of attenuated micelles that constitute the gelatinous coat of the egg. Antisera that are prepared against purified fertilizin, derived from the gelatinous coat, may be expected to react with the plasma membrane of the fertilized (deprived of vitelline membrane) as well as the unfertilized egg. It is, then, understandable that such antisera can block development of the fertilized egg. In fact the evidence(5) shows that antibodies directed against internal constituents of the egg (*e.g.*, prepared by absorbing antiwhole egg antisera) exert very little if any cytotoxic action on the intact unfertilized, or fertilized, egg.

In the previously reported(1-3) studies there was evidence that the heat-labile components (C'1 and C'2) of complement are not needed for the cleavage-blocking action of the antisera *vs* fertilizin since such sera remained active after being heated to 56°C for ½ hour. Since, however, complement has

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