COENZYME Q AND THE STABILITY OF BIOLOGICAL MEMBRANES

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We have recently observed that anemic and dystrophic monkeys develop reticulocytosis on treatment with a normally synthesized body component, coenzyme Q_{10} (Fitch, et al., 1965). Complete remission of the anemia is effected by hexahydrocoenzyme Q_4 therapy (Fitch, et al., 1965). The anemia, which develops in monkeys deprived of vitamin E, also responds well to vitamin E-treatment (Dinning and Day, 1957). This apparent connection between an intrinsic coenzyme and a vitamin has been studied by observing the ability of hexahydrocoenzyme Q_4 to protect red blood cells from lysis by H_2O_2. This study was prompted by two facts: (a) vitamin E protects cells and subcellular particles from lysis by oxidants (Rose and György, 1952; McKnight, Hunter, and Oehlert, 1965), and (b) coenzyme Q is present in the lipoprotein complexes of cellular membranes (Green, 1965). We found that hexahydrocoenzyme Q_4 and the 6-chromanol of hexahydrocoenzyme Q_4 protect erythrocytes of premature infants from hemolysis by H_2O_2.

METHODS

The susceptibility of red blood cells to lysis was measured by the method of György, Cogan, and Rose (1952) as modified by Gordon, Nitowsky, and Cornblath (1955)--except that a one-hour preincubation with one of the substances listed in Table I always preceded the incubation with H_2O_2. Erythrocytes were

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obtained from the femoral vein of premature infants ranging in age from 4 to 57 days. The compounds were originally suspended at a concentration of 10 mg per ml in a 5% glucose solution containing 10% Emulphor (EL 620) and 5% N,N-dimethylacetamide as dispersants and 0.004% merthiolate as perservative. Dilutions of the original suspensions were prepared with 0.9% NaCl solution. In the amounts used, the suspending vehicle did not affect the susceptibility of the erythrocytes to hemolysis by H_2O_2. For preincubation, a 5% suspension of washed erythrocytes was prepared in a 0.9% NaCl solution containing the test compound, and the mixture was incubated for one hour at 37°. The supernatant fluid was then discarded and a final 5% suspension of erythrocytes was prepared in 0.9% NaCl solution. To test susceptibility to hemolysis, a 1:1 mixture of the final 5% erythrocyte suspension and 2.4% H_2O_2 in phosphate buffer (25 ml of 0.2 M KH_2PO_4 plus 19.7 ml of 0.2 M NaOH diluted to 100 ml with distilled water; pH 7.4) was incubated for 15 minutes at 37° and then for 165 minutes at room temperature. Each test of susceptibility to H_2O_2 was done in duplicate and the average values are shown. In this test, red blood cells from normal individuals are stable (less than 5% hemolysis) and red blood cells from premature infants are unstable.

Table I

HEMOLYSIS OF RED BLOOD CELLS BY H_2O_2

Addition to preincubation mixture compound	concentration	Individual patients A	B	C	D	E	F	G	H
	µg/ml	% Hemolysis							
suspending vehicle		79	47	68	68	90	55	22	75
d-α-tocopherol	1.0	64	13	1	75	87	14	10	53
d-α-tocopherol	10.0	-*	--	--	2	51	3	2	17
6-chromanol of Q_4 [+]	1.0	--	--	--	--	51	2	3	20
hexahydrocoenzyme Q_4	10.0	41	33	10	19	51	8	4	51
d-α-tocopherylquinone	10.0	--	--	27	41	--	--	--	67
trimethylphytyl-benzoquinone [‡]	10.0	--	--	--	--	--	--	16	63

*The dash means not tested.
[+]6 chromanol of hexahydrocoenzyme Q_4
[‡]2,3,5-trimethyl-6-phytyl-1,4-benzoquinone

RESULTS AND DISCUSSION

The 6-chromanol of hexahydrocoenzyme Q_4 was most effective in protecting red blood cells against H_2O_2; one microgram was equal in effect to 10 micrograms of d-α-tocopherol. Although this chromanol is not found in nature, its effectiveness is ascribed to membrane sites that prefer the methoxy-group nucleus of coenzyme Q over the methyl-group nucleus of α-tocopherol. Animals are known to possess enzymes that require the methoxy-group nucleus of coenzyme Q (Folkers, et al., 1966).

Since the intrinsic coenzyme Q of membranes is in the quinone⇌hydroquinone forms, more importance can be attached to the finding that a quinone, hexahydrocoenzyme Q_4, prevents hemolysis (Table I). This compound is a full substitute for native coenzyme Q_{10} in the succinoxidase system (Folkers, et al., 1966); and by stabilizing a biological membrane, it presumably exhibits another activity common to natural coenzyme Qs. In support of this presumption, Lucy and Dingle (1964) found that coenzyme Q_6 protects normal red blood cells from lysis by excess vitamin A. One way by which coenzyme Q could protect membranes is demonstrated by the report of Mellors and Tappel (1966) showing that the hydroquinone form of coenzyme Q_6 protects mitochondrial lipids from peroxidation.

In limited tests of specificity, d-α-tocopherylquinone and 2,3,5-trimethyl-6-phytyl-1,4-benzoquinone provided some, albeit poor, protection. This finding agrees with the work of Nitowsky and Tildon (1956) showing that other quinones protect red blood cells of infants from H_2O_2. Clearly, the stability of biological membranes in vitro can be improved by compounds other than vitamin E and coenzyme Q. Nevertheless, if a quinone⇌hydroquinone normally protects membranes in vivo, coenzyme Q would be the one most likely to serve such a role in animals.

We propose that coenzyme Q does protect membranes in vivo. Further, we suggest that the need for an intrinsic compound to stabilize membranes increases with increasing vitamin E deficiency and that, to fill the increased need, coenzyme Q_{10} is diverted from its role in the electron transport chain.

Eventually this unusual demand would exceed the animal's capacity to supply coenzyme Q for critical uses in the developing red blood cell. Only then would the anemia of vitamin E deficiency develop.

An insufficient concentration of coenzyme Q_{10} to permit maturation of the red blood cell would explain both the anemia of vitamin E deficiency and its response to coenzyme Q_{10} treatment.

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