tility, an area previously thought to be beyond the realm of mass spectrometry.

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THE RESPONSE OF THE ANEMIC AND DYSTROPHIC MONKEY TO TREATMENT WITH COENZYME Q

Sir:

The anemia and muscular dystrophy that develop in rhesus monkeys (*Macacus mulatta*) supplied with a purified diet low in vitamin E is rapidly progressive, and has been found to lead to death invariably unless α-tocopherol is given.[1] The specific metabolic defect(s) responsible for these abnormalities remains unknown, although various alterations in metabolism in the deficient monkey have been observed.[1-3] These metabolic deficiencies resulted after maintenance of the monkey for many months, and the deficiencies and the response to α-tocopherol naturally were interpreted solely on the basis of vitamin E. Most of these studies were carried out before the discovery and elucidation of coenzyme Q (ubiquinone).

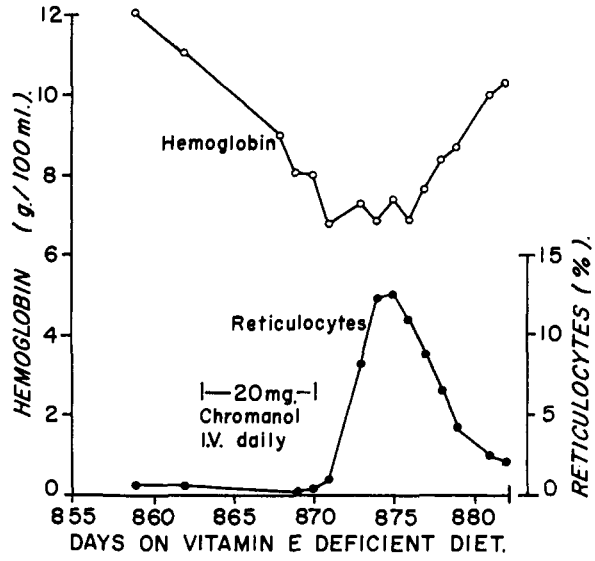


Fig. 1.—The response of a deficient, anemic monkey (no. 223) to the 6-chromanol of hexahydrocoenzyme Q_4.

Today, new knowledge is available on the isolation of coenzyme Q from natural sources, and on the role of coenzyme Q with succinoxidase and cellular respiration. When one compares the organic structures of certain members of the vitamin E and coenzyme Q groups, such as α-tocopherol (I) and the 6-chromanol[4] of hexahydrocoenzyme Q_4 (II), it is seen that they are identical, except for the interchange of two methyl and two methoxy groups in the 7 and 8 positions. Although only quinones of the coenzyme Q group (coenzyme Q_{10} has been isolated from tissue of the rhesus monkey)[5]

(1) J. S. Dinning and P. L. Day, *J. Exp. Med.*, **105**, 395 (1957).
(2) J. S. Dinning and P. L. Day, *J. Nutr.*, **63**, 393 (1957).
(3) J. S. Dinning and P. L. Day, *J. Biol. Chem.*, **233**, 240 (1958).
(4) C. H. Shunk, N. R. Trenner, C. H. Hoffman, D. E. Wolf, and K. Folkers, *Biochem. and Biophys. Res. Comm.*, **2**, 427 (1960).
(5) Unpublished data of Gale, Page and Folkers.

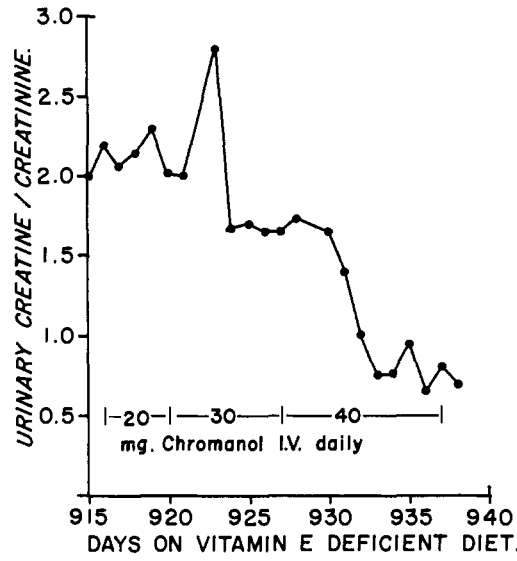
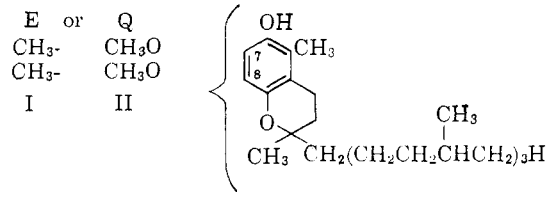


Fig. 2.—The response of a dystrophic deficient monkey (no. 218) to the 6-chromanol of hexahydrocoenzyme Q_4.

have been characterized from tissue extracts to date, their reductively cyclized chromanols have already been shown to have biological activity, and are being studied as possible enzymic forms of coenzyme Q. Two examples of comparable biological



activity of α-tocopherol and members of the coenzyme Q group are fetal resorption in rats[6] and the maintenance of chick sperm motility.[7] On the basis of such activities in both the E and Q groups, the known biochemistry of coenzyme Q as well as related organic structures, we have studied the responses of the anemic and dystrophic monkey to the treatment with the 6-chromanol of hexahydrocoenzyme Q_4 (II), as the first Q-form compound to test.

The handling of the animals and the purified diets, which are deficient of vitamin E, have been described.[1,8] Two animals were used. One (No. 223) received a low-fat diet and the other (No. 218) received a diet that contained 8% of lard, and was supplemented with 3 g. of cod liver oil daily. Both diets were supplied *ad libitum*. The first monkey had one prior test with α-tocopherol, and a good response was obtained. In the monkey that received fat in the diet, only a partial remission (as usual) had been obtained with N,N'-diphenyl-*p*-phenylenediamine. Each monkey was in a definite relapse and appeared near death before treatment with II. They were injected intravenously with a

(6) B. C. Johnson, Q. Crider, C. H. Shunk, B. O. Linn, E. L. Wong, and K. Folkers, *Biochem. and Biophys. Res. Comm.*, **5**, 309 (1961).
(7) A. C. Page, Jr., M. C. Smith, P. H. Gale, D. Polin, and K. Folkers, *Biochem. and Biophys. Res. Comm.*, **6**, 141 (1961).
(8) C. D. Fitch, J. S. Dinning, L. A. Witting and M. K. Horwitt, *J. Nutr.*, **75**, 409 (1961).

solution containing 10 mg. per ml. of II suspended in water with emulphor and DMA as dispersants.

Both monkeys had an unequivocally favorable clinical response to the 6-chromanol of hexahydrocoenzyme Q_4. In addition, there was a pronounced reticulocyte response in the anemic monkey that was followed by an increase in hemoglobin (Fig. 1). The excretion of creatine was sharply reduced in both animals, as in Fig. 2 for No. 218. This response is similar to that obtained when α-tocopherol is given.[1] A dose of 20 mg. of II given intravenously daily for 8 days produced a remission in the monkey supplied with the low-fat diet, but 40 mg., given intravenously daily for an extended period, was required to keep the creatine to creatinine ratio below 1 in the monkey receiving fat in the diet.

Although these studies were done in only two animals, they had received extensive over-all study, and the response appears clearly due to the administration of the 6-chromanol of hexahydrocoenzyme Q_4. Remissions, such as those reported here, have not occurred previously without specific therapy. The interpretation of this finding may be based upon answers to the following questions: (a) Are both vitamin E and the chromanol of coenzyme Q_4 exhibiting a basic biological activity in the deficient monkey in the sense that each is functioning in a native biochemical sequence? (b) Is only vitamin E or the chromanol of coenzyme Q_4 exhibiting a basic biological activity with the other substituting solely on the basis of similarity of structure? (c) Is one compound merely protecting the other, because of its antioxidant properties?

After about twenty-five years of research on vitamin E, a direct relationship between it and a given disease state in man is not generally recognized in medicine. Horwitt[9] has investigated man's need for tocopherol as a function of peroxidizable lipids in the diet. Coenzyme Q_{10} is present in man.[10] That it, or other enzymic forms, may show effects in man not seen with vitamin E requires new medical research which may be further justified by these new data on the response of the anemic and dystrophic monkey to therapy with II.

(9) M. K. Horwitt, *Am. J. Clin. Nutr.*, **8**, 451 (1960).

(10) P. H. Gale, F. R. Koniuszy, A. C. Page, Jr., and K. Folkers, *Arch. Biochem. Biophys.*, **93**, 211 (1961).

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* Coenzyme Q. XXXIII.

SYNTHESIS OF SOME STABILIZED 2,3-NAPHTHOQUINONOID SYSTEMS

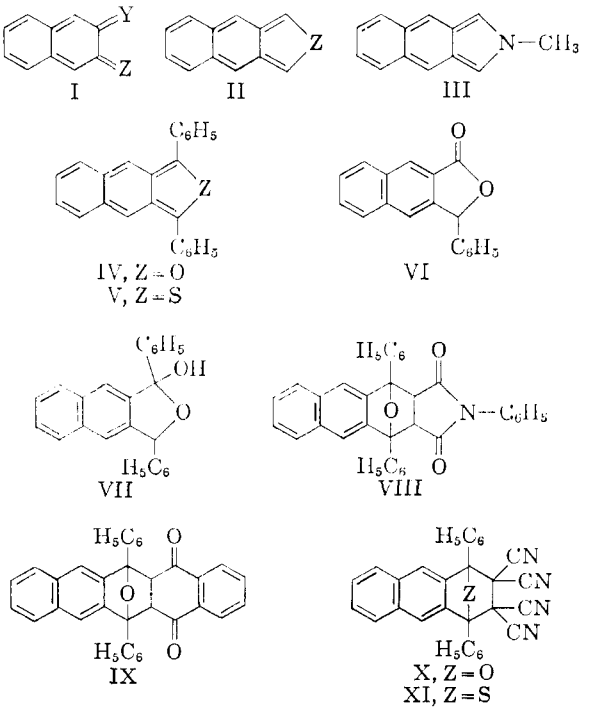
Sir:

With the exception of anthracene and higher fused linear polynuclear aromatic hydrocarbons, no compounds of the type I or II have been reported. Substances of this sort may be considered to be formally related to the unknown 2,3-naphthoquinone (I, Y = Z = O), a high energy system containing no true benzenoid ring. A recent attempt to synthesize 2-methyl-5,6-benzisoindole (III), a system of type II, was not successful; the product, if formed, was too unstable to permit isolation or even trapping by maleic anhydride.[1] We wish now to report the synthesis of furan IV and thiophene V, both of which represent isolable 2,3-naphthoquinonoid systems.

Reduction of 3-benzoyl-2-naphthoic acid[2] with sodium borohydride, then acidification, gave the lactone VI, m.p. 153–155°, in 80% yield.[3] Reaction of VI with excess phenylmagnesium bromide gave, in 65% yield, alcohol VII, m.p. 153–156°. Brief heating of VII with acetic acid gave, in 88% yield, 1,3-diphenylnaphtho[2,3-c]furan (IV), m.p. 148–154°, as small dark red plates: $\lambda_{max}^{benzene}$ 367 mμ (ϵ = 5700), 383 (5700), 524 (7900), 546 (7900).

In the absence of light, compound IV was unchanged after standing at room temperature for several months. In solution it is much less stable, and all attempts at recrystallization have failed. Substance IV is extraordinarily reactive as a diene in the Diels–Alder reaction. It reacts instantaneously in solution with N-phenylmaleimide, 1,4-naphthoquinone and tetracyanoethylene to give excellent yields of the corresponding adducts VIII (m.p. 287–290°), IX (m.p. 198–203°) and X (m.p. 235–258°). Adducts IX and X, however, can reverse to their precursors with surprising ease: solutions of these colorless compounds in benzene become red on heating and lose their color once more when cooled.

Reaction of IV with phosphorus pentasulfide in disulfide for one day gave, in 38–61% yield, 1,3-diphenylnaphtho[2,3-c]thiophene (V), isolated as its deep purple 2,4,7-trinitrofluorenone complex, m.p. 169–171°. The free thiophene (V), obtained



(1) G. Wittig and H. Ludwig, *Ann.*, **589**, 55 (1954).

(2) H. Waldmann and H. Mathiowitz, *Ber.*, **64**, 1713 (1931).

(3) Melting points are uncorrected. Satisfactory analyses were obtained for all compounds whose melting points are recorded.