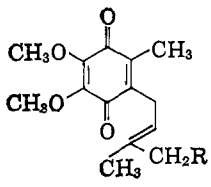
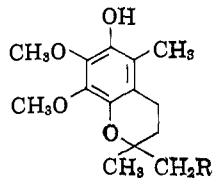
Coenzyme Q. LVI. Novel Cyclization of Quinones to Chromanols and Possible Iron–Coenzyme Q Complexes[1]

Sir:

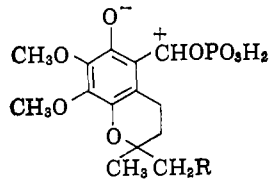
Coenzyme Q_{10} (I) is cyclized to the corresponding chromanol (II) by the action of either ferric or cupric chloride and L-ascorbic acid in glacial acetic acid; this novel procedure has other significant implications in the chemistry of CoQ and vitamins E and K. Previous cyclizations[2,3] of CoQ to chromanols frequently also resulted in an extensive and undesirable cyclization of the unsaturated side chain. This new procedure is of general applicability and makes many chromanols of the CoQ and vitamins E and K groups with unsaturated polyisoprene side chains available for biological research: the cyclization of the side chain is under far better control, but is still a side reaction. It appears that this conversion may have direct relationship to the interaction and stoichiometry of coenzyme Q and nonheme iron in the electron-transport chain.



I



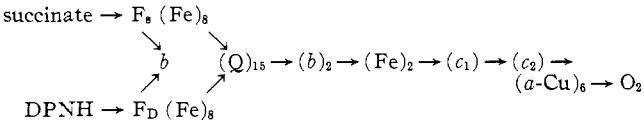
II



III

The biological and medical importance of the chromanols in the coenzyme Q group has been demonstrated. Children with anemia,[4,5] anemic and dystrophic monkeys,[6] dystrophic rabbits,[7] encephalomalacia in chicks,[8] and the resorption–gestation syndrome in rats[8] have been therapeutically treated by administration of coenzyme Q_4–chromanol. The chromanol of coenzyme Q_{10} is of particular interest since coenzyme Q_{10} is present in most animal and human tissue.

Coenzyme Q is assigned by Green[9] a position in the respiratory electron-transfer chain between flavoprotein iron and cytochrome *b*. The organic structural bonding of this nonheme iron in the chain is unknown. We have noted the iron–CoQ stoichiometry of 8:15 by Green and Wharton,[10] and consider that an iron–coenzyme Q chelate of 1:2 molecules is indicated. On this basis, the true stoichiometry of this detail of the chain would be $(Fe)_7$, $(Fe)_8$, etc., and $(Q)_{14}$, $(Q)_{16}$, etc., so that the ratio is 1:2, and this ratio is permissive when one considers the limits of analysis. It is known that phosphate readily coordinates to iron, and a vitamin E quinone–nickel complex has been studied.[11]



It appears to us that the "nonheme" iron, associated with F_S and F_D, may be a CoQ–Fe chelate (IV) in a macromolecular lipid–protein (coupling factor)[12] complex which functions in the electron-transfer process through its overlapping orbitals.

This CoQ–Fe chelate (IV) is proposed for the electron-transport chain and quite apart from the interest in a coupled role for CoQ in phosphorylation. In regard to a possible role for CoQ in the biosynthesis of ATP, structure III was proposed for "active phosphate." When the functionality of III and the herein described novel cyclization of CoQ_{10} to its chromanol by Fe^{+3} are considered in conjunction with the CoQ–Fe chelate (III), then we extend the phosphorylation mechanism, IV → VII → IV, to give ATP.

The CoQ–Fe chelate (IV) could function in the respiratory electron transfer independently of a coupling mechanism. In coupling, structures V and VI represent a proton-catalyzed isomerization of one quinone moiety in IV to the methine in V; next, reaction of the methine with P_i gives the phosphate (VI). Formation of V and VI is independent of electron transfer. There are two internal electron transfers reversibly between the two nuclei of VI and VII with concomitant reaction of VII with ADP yielding ATP and V. One choice of resonance structure exemplifies (Fig. 1) the principle of a 2CoQ–Fe chelate, as well as the choice of steric preference of group orientation on the two quinone nuclei.

The 2CoQ–Fe chelate is not inconsistent with studies on the DPNH–CoQ phosphorylation site[13] or on the nonheme iron protein of the reduced CoQ–cytochrome *c* reductase complex[14,15]; these latter studies used techniques which could degrade a CoQ–Fe chelate.

The concept of the CoQ–Fe chelate indicates that an *in vivo* deficiency of *either* CoQ or Fe could mean a deficiency at the chelate site of *both* CoQ and Fe. Treatment of human disease with either iron (*e.g.*, hypochromic anemia) or CoQ[5] (or vitamin E)[16] might be improved by treatment with both entities to reconstitute the chelate; support for such combination therapy is apparent in the finding[17] that hemoglobin levels in rats were better when iron had been given with

(1) This research was partially supported by funds from the Merck Sharp and Dohme Research Laboratories and we express our appreciation to Dr. Max Tishler.

(2) C. H. Hoffman, N. R. Trenner, D. E. Wolf, and K. Folkers, *J. Am. Chem. Soc.*, **82**, 4744 (1960).

(3) C. H. Shunk, N. R. Trenner, C. H. Hoffman, D. E. Wolf, and K. Folkers, *Biochem. Biophys. Res. Commun.*, **2**, 427 (1960).

(4) A. S. Majaj, J. S. Dinning, and K. Folkers, Congress of the European Society of Hematology, Lisbon, Aug. 26–30, 1963.

(5) J. S. Dinning, A. S. Majaj, S. A. Azzam, W. J. Darby, C. H. Shunk, and K. Folkers, *Am. J. Clin. Nutr.*, **13**, 169 (1963).

(6) J. S. Dinning, C. D. Fitch, C. H. Shunk, and K. Folkers, *J. Am. Chem. Soc.*, **84**, 2007 (1962).

(7) A. F. Wagner, R. J. Stopkie, and K. Folkers, *Arch. Biochem. Biophys.*, in press.

(8) J. L. Smith, H. N. Bhagavan, R. B. Hill, S. Gaetani, P. B. Rama Rao, Q. E. Crider, B. C. Johnson, C. H. Shunk, A. F. Wagner, and K. Folkers, *ibid.*, **101**, 388 (1963).

(9) D. E. Green in "Quinones in Electron Transport," Little, Brown and Company, New York, N. Y., 1961, p. 130.

(10) D. E. Green and D. C. Wharton, *Biochem. Z.*, **338**, 335 (1963).

(11) G. N. Schrauzer, *Angew. Chem. Intern. Ed. Engl.*, **1**, 404 (1962).

(12) R. L. Prairie, T. E. Conover, and E. Racker, *Biochem. Biophys. Res. Commun.*, **10**, 422 (1963).

(13) M. Hansen and A. L. Smith, *Biochim. Biophys. Acta*, **81**, 214 (1964).

(14) J. S. Rieske, D. H. MacLennan, and R. Coleman, *Biochem. Biophys. Res. Commun.*, **15**, 338 (1964).

(15) R. Coleman, J. S. Rieske, and D. Wharton, *ibid.*, **15**, 345 (1964).

(16) J. S. Dinning, A. S. Majaj, S. A. Azzam, and W. J. Darby, *Federation Proc.*, **22**, 550 (1963).

(17) S. M. Greenberg, R. G. Tucker, A. E. Heming, and J. K. Mathues, *J. Nutr.*, **63**, 19 (1957).

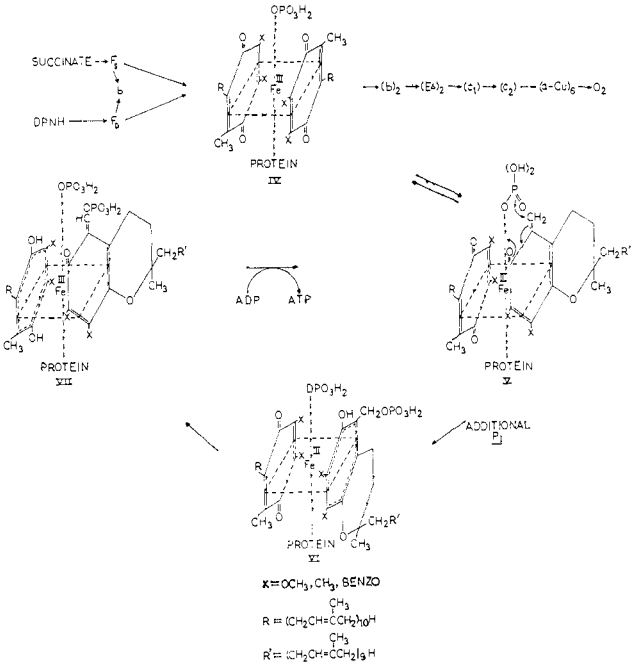


Fig. 1.—Macromolecular lipid–protein complex.

vitamin E and ascorbic acid than if given alone or with either vitamin separately.

Coenzyme Q_{10} was dissolved in glacial acetic acid containing an equimolar amount of ferric chloride and a fivefold excess of L-ascorbic acid. This solution was refluxed for approximately 1.5 hr. and then poured into water. The aqueous mixture was extracted with *n*-hexane. The hexane extract was washed several times with water, dried over anhydrous sodium sulfate, and then concentrated *in vacuo*, leaving a viscous yellow-brown oil. Thin layer chromatography on silica gel G (30% ether in *n*-hexane) showed only one Emmerie-Engel sensitive spot at R_f = 0.44. A sample of this material was further purified by preparative thin layer chromatography. The 60-Mc. n.m.r. spectrum of the 6-chromanol of CoQ_{10} in carbon tetrachloride is the best criterion of its identity; τ 4.88 (OH), 4.99 (CH=), 6.16 and 6.28 (CH_3O–), 7.48 triplet (—CH_2C=), and 8.01–9.1 (saturated alkyl groups). The infrared absorption spectrum of the product is nearly identical with the spectrum obtained from a sample of the chromanol of hexahydrocoenzyme Q_4. The major bands in the spectrum are 3480 (OH), 2900 (C–H stretching), 1450 (C–H deformation), and 1170 and 1190 cm.$^{-1}$ (C–O–CH_3).

Acknowledgment.—Appreciation is expressed to Dr. David E. Green of the University of Wisconsin for his continuing emphasis on the importance of nonheme iron in the chain.

STANFORD RESEARCH INSTITUTE
MENLO PARK, CALIFORNIA
HAROLD W. MOORE
KARL FOLKERS

RECEIVED JUNE 4, 1964

Radical Exchange during Nitrite Photolysis[1]

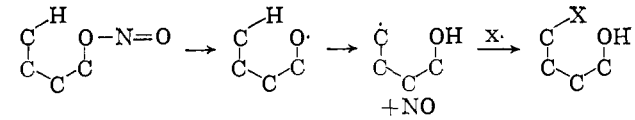
Sir:

Recent examination of nitrite ester photolysis[2] with intramolecular hydrogen transfer has established[3] the mechanism (Scheme I) indicated below (X = NO).

(1) Communication No. 31 from the Research Institute for Medicine and Chemistry.

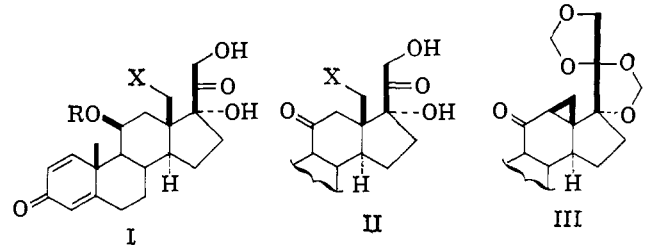
(2) D. H. R. Barton, J. M. Beaton, L. E. Geller, and M. M. Pechet, *J. Am. Chem. Soc.*, **82**, 2640 (1960); **83**, 4076 (1961).

SCHEME I



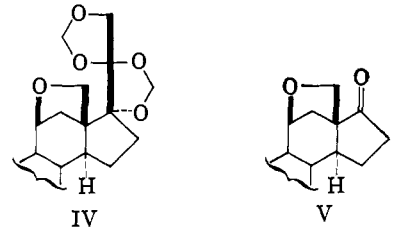
It seemed clear to us that other radicals (X·) could compete with NO and that radical transfer would also be possible. We now report examples of such radical-exchange reactions.

Photolysis[2] of prednisolone 21-acetate 11-nitrite[4] (Ia, R = NO, X = H) in benzene containing iodine (1.2 moles) gave 18-iodoprednisolone acetate (Ia, R = H, X = I; 28%), m.p. 131–134° dec., $[\alpha]_D$ +93° (all $[\alpha]_D$ *c* 1, chloroform), λ_{max}^{MeOH} 242 mμ (ε 15,300). Oxidation with chromic acid in acetone gave 18-iodoprednisone acetate (IIa, X = I), m.p. 172–176° dec., $[\alpha]_D$ +138°, λ_{max}^{MeOH} 239 mμ (ε 13,200). Similar photolysis of dexamethasone 21-acetate 11-nitrite[4] (Ib, R = NO, X = H) gave 18-iododexamethasone 21-acetate (Ib, R = H, X = I; 37%), m.p. 140–143° dec., $[\alpha]_D$ +89°, λ_{max}^{EtOH} 236 mμ (ε 12,600), oxidized as above to the 11-ketone IIb (X = I), m.p. 145–150° dec., $[\alpha]_D$ +125°, λ_{max}^{EtOH} 233 mμ (ε 15,000). Similar photolysis and oxidation of 17α,20;20,21-bismethylenedioxyprednisolone 11-nitrite (Ic, R = NO, X = H) gave 17α,20;20,21-bismethylenedioxy-18-iodoprednisone (IIc, X = I), m.p. 186–189° dec., $[\alpha]_D$ +40°, λ_{max}^{MeOH} 239 mμ (ε 16,200). Treatment of IIc (X = I) with potassium hydroxide in methanol gave the 12β,18-cyclopropyl ketone III,[5] m.p. 288–289°, $[\alpha]_D$ −21°, λ_{max}^{MeOH} 240 mμ (ε 16,400), which regenerated IIc (X = I) when treated with hydrogen iodide. Treatment of the initial photolysis product of Ic (R = NO, X = H) with methanolic potassium acetate gave the 11β,18-oxide IV (23%), m.p. 255–260°, $[\alpha]_D$ +5°, λ_{max}^{MeOH} 242 mμ (ε 16,200). Removal of the methylenedioxy groups and oxidation with sodium bismuthate gave 11β,18-oxidoandrosta-1,4-diene-3,17-dione, m.p. 168–170°, $[\alpha]_D$ +190°, λ_{max}^{MeOH} 242 mμ (ε 15,100), also obtained by photolysis of 11β-hydroxyandrosta-1,4-diene-3,17-dione nitrite[6] in the presence of iodine.



I II III

a, 21-acetate; b, 9α-fluoro-16α-methyl 21-acetate; c, 17α,20;20,21-bismethylenedioxy



IV V

(3) M. Akhtar and M. M. Pechet, *ibid.*, **86**, 265 (1964).

(4) M. Akhtar, D. H. R. Barton, J. M. Beaton, and A. G. Hortmann, *ibid.*, **85**, 1512 (1963).

(5) *Cf.* J. F. Kerwin, M. E. Wolff, F. F. Owings, B. B. Lewis, B. Blank, A. Magnani, C. Karash, and V. Georgian, *J. Org. Chem.*, **27**, 3628 (1962).

(6) H. Reimann, A. S. Capomaggi, T. Strauss, E. P. Oliveto, and D. H. R. Barton, *J. Am. Chem. Soc.*, **83**, 4481 (1961).