toration of activity. Addition of zinc in excess of 1 gram atom per mole does not result in additional activity and may inhibit; this excess zinc can be removed by dialysis at neutral *p*H. Similar results are obtained when the zinc is removed at *p*H 4.2, in the presence or absence of OP, and the protein further treated as above.

Dialysis of the metal-free protein against ions of Group IIA and IIB elements—Mg^{++}, Ca^{++}, Cd^{++}, and Hg^{++}—did not restore activity.

On the other hand, dialysis of the metal-free protein against ions of the first transitional period—Cr^{+++}, Mn^{++}, Fe^{++}, Co^{++}, and Ni^{++}—resulted in a significant *restoration* of activity.

The present experiments indicate the crucial nature of the metal atom as an active component of the enzymatic site of this exopeptidase. Carboxypeptidase can be isolated with its full complement of zinc, thus differing from many metal activated systems. This zinc can be successively removed and then be replaced. Therefore, carboxypeptidase constitutes a suitable model system to explore the significant difference in the physical-chemical and enzymatic properties of *metalloenzymes* and *metal-enzyme-complexes*.[6]

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BIOPHYSICS RESEARCH LABORATORY OF THE DEPARTMENT OF MEDICINE
HARVARD MEDICAL SCHOOL AND THE PETER BENT BRIGHAM HOSPITAL
BOSTON, MASSACHUSETTS
DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF WASHINGTON
SEATTLE, WASHINGTON

BERT L. VALLEE
JOHN A. RUPLEY
THOMAS L. COOMBS
HANS NEURATH

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COENZYME Q: A NEW GROUP OF QUINONES[1]

Sir:

The isolation of crystalline quinone (Q-275) (I) from lipides of beef heart mitochondria has been described.[2,3,4] I acts as a coenzyme by undergoing cyclic oxidation and reduction during substrate oxidation in mitochondria.[2,5,6] Evidence bearing on the possible role of I in oxidative phosphorylation also has been presented.[7,8] *Anal.* Found: C, 82.24 ± 0.32 (8); H, 10.38 ± 0.27 (8); O, (Unterzaucher) 7.60, 7.71; O-Me, (Zeisel) 6.78 ± 0.16 (6); C-Me, (Kuhn-Roth) 13.4 ± 2.1 (3); moles H_2 absorbed per 100 g., 1.29; acetyl, zero; equiv. wt., 447; mol. wt., 900 and 910 (X-ray diffraction[9]), 779 (isothermal distillation), 527, 632, and 697 (ebullioscopic in butanone, acetic acid, ethanol, respectively); m.p. 49.9°; λ_{max}.275 mμ, 405 mμ, ethanol (oxidized form) and λ_{max}.290 mμ, ethanol (reduced form)[2]. By reductive acetylation the diacetate of dihydro I (m.p. 40°) (II) was prepared (*Anal.* Found: C, 79.41; H, 9.71; O-Me, 6.50, 7.08; acetyl, 9.96; moles H_2 absorbed per 100 g., 1.05; 1000 [X-ray], 1100 (isothermal distillation)). Levulinic acid, acetic acid and succinic acid have been identified as products of alkaline potassium permanganate treatment of I. A paraquinone function is indicated by the spectra of I and II. The other two oxygens are accounted for by two alkoxyl groups. A polyisoprenoid side chain(s) with one double bond per unit is indicated by the oxidation products and hydrogenation. X-Ray analysis[9] shows a pseudo-periodicity which is expected for such a side chain. The consumption of 11.1 moles of hydrogen, based on a molecular weight of 863,[10] indicates ten mono-unsaturated isoprenoid units.

This work has been confirmed and extended in accompanying communications.[10,11]

We also have succeeded in isolating from microbial sources four[12] other crystalline compounds which we believe to be homologs of I, differing from each other in the length of their polyisoprenoid side chains. On the basis of the formula $C_{59}H_{90}O_4$[10] for I, these compounds are formulated as III, $C_{54}H_{82}O_4$, Torula yeast, m.p. 45.2°; IV, $C_{49}H_{74}O_4$, *A. vinelandii*, m.p. 37°; V, $C_{44}H_{66}O_4$, Torula yeast, m.p. 30.5°; VI, $C_{39}H_{58}O_4$, *S. cerevisiae*, m.p. 16°. All data available support these conclusions. The ultraviolet and visible absorption spectra of all these compounds (I, III, IV, V, VI) are qualitatively identical, indicating a common quinonoid chromophoric group; equivalent weight (alkoxyl, oxygen, oxidation-reduction titration of hydroquinone) and molecular weight determinations (X-ray) confirm that these compounds differ from the next lower homologue by a five-carbon isoprenoid unit. In particular, cell dimensions[9] of I, III, IV substantiate this point. The estimate of the number of isoprenoid groups based on hydrogenation data would be 9.4, 8.0, 7.0 for III, IV and V, respectively.

Since all five quinones have coenzymatic activity,[5] we have altered our designation Q-275 to coenzyme Q to represent this new group of compounds. On the basis of the apparent number of isoprenoid units, we have designated the individual members of the coenzyme Q group as coenzyme Q_{10} (I); coenzyme Q_9 (III); coenzyme Q_8 (IV); coenzyme Q_7 (V), coenzyme Q_6 (VI).

Ubiquinone (SA) was described by Morton, *et al.*[13-16] as preparations (m.p. range 33-41°) from

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animal organs and yeast (oily fractions). It is clearly related to our coenzyme Q group.

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CONTRIBUTION FROM
INSTITUTE FOR ENZYME RESEARCH
THE UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

R. L. LESTER
F. L. CRANE
Y. HATEFI

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COENZYME Q. I. STRUCTURE STUDIES ON THE COENZYME Q GROUP

Sir:

The discovery of a quinone, Q-275, has been reported,[1] and characterizing chemical and physical properties, oxidative degradation and hydrogenation are reported in an accompanying communication.[2] Four closely related quinones having similar coenzymatic activity have been isolated from microbial sources.[2] All five new quinones are designated as members of a coenzyme Q group, *i.e.*, coenzyme Q_6, Q_7, Q_8, Q_9 and Q_{10}.

We now have extended these observations. Formula I agrees with our structural data for coenzyme Q_{10} (beef heart) and II and III correspond to Q_9, Q_8, etc.

CH_3O, CH_3O, CH_3, $(CH_2CH{=}C(CH_3){-}CH_2)_n H$ on benzoquinone ring — I, $n = 10$; II, $n = 9$; III, $n = 8$, etc.

Our isolated Q_{10} (yellow) melted at 49.5–50.5°. Found: C, 81.98, 82.05; H, 10.38, 10.31. Hydrogenation of Q_{10} resulted in an absorption of about 11 moles of hydrogen. Oxidation of the resulting hydroquinone yielded eicosahydro-coenzyme Q_{10}; $\lambda_{max.}^{isooctane}$ 278 mμ, $E_{1 cm.}^{1\%}$ 187. Found: C, 80.30; H, 12.36.

Proton type	C.p.s.[a]	Relative band areas	No. of protons based on $2CH_3O$/mole	No. of protons calcd. for $C_{59}H_{90}O_4$ and structure I
$\underline{H}C{=}$	+8	5	10	10
$C\underline{H}_3O{-}$	−34	3	6	6
$=C{-}C\underline{H}_2{-}CH{=}$	−64, −69	1	2	2
$=C{-}C\underline{H}_2{-}C\underline{H}_2{-}C{=}$ / $C\underline{H}_3C{=}$(nucleus)	−113	20	40	36 / 3
$C\underline{H}_3C{=}$(chain)	−125	16.5	33	33
			91	90

[a] The bands refer to 40 mc. spectra in carbon tetrachloride, + means at lower fields than water protons while − means at higher fields.

Coenzyme Q_{10} appears to be a 2,3-dimethoxybenzoquinone derivative. Its absorption spectrum with maxima at 275 mμ and 405 mμ is in good agreement with that of aurantiogliocladin, (maxima at 275 and 407 mμ) identified as 2,3-dimethoxy-5,6-dimethylbenzoquinone[3].

We have studied the nuclear magnetic resonance spectra at 40 mc. of Q_{10} and many synthetic model compounds, and Dr. James N. Shoolery (Varian Associates) has kindly determined the spectrum of Q_{10} at 60 mc. These data characterize the protons of Q_{10}: these data show convincingly that Q_{10} has two $CH_3O{-}$, one $CH_3{-}$ and one isoprenoid chain of 10 units attached to a benzoquinone nucleus, but do not define the position of the ring substituents. N.m.r. data exclude the presence of aromatic proton, $-\overset{|}{C}{=}C\underline{H}_2$ and $-C\underline{H}_2C\underline{H}_3$, and assign the ten double bonds in the isoprenoid side chain as in I. The n.m.r. spectrum of eicosahydrocoenzyme Q_{10} shows the one $=CC\underline{H}_3$ nucleus group at −111.5 c.p.s. While this group is not clearly resolved from $=C{-}CH_2C\underline{H}_2C{=}$ in the spectrum of Q_{10} at 40 mc., it is at 60 mc.

Reduction and methylation of Q_{10} with dimethyl sulfate, gave a colorless crystalline tetramethoxy derivative (IV), m.p. 38-39°, $[\alpha]_D$ 0° (chloroform). Found: C, 81.85; H, 10.82, OCH_3, 14.2.

Oxidation of IV with about a 4-fold excess of aqueous alkaline permanganate at 100° yielded tetramethoxyphthalic anhydride (V) (after sublimation), m.p. 138–139° not depressed by admixture of synthetic tetramethoxyphthalic anhydride.

V: tetramethoxyphthalic anhydride (CH_3O ×4, anhydride ring). VI: CH_3O ×4, CH_3, CH_2CO_2H on benzene ring.

Oxidation of IV in acetone with the stoichiometric quantity of permanganate for ten double bonds gave after partition chromatography, 2-methyl-3,4,5,6-tetramethoxyphenylacetic acid (VI) m.p. 75-76°, identical by melting point behavior and infrared spectra with a synthetic sample. The presence of levulinic and succinic acids as oxidation products was confirmed.[2]

The chemical and physical properties of synthetic 2,3-dimethoxy-5-methyl-6-farnesylbenzoquinone[4] and analogs[4] are closely similar to those of Q_{10}. Ubiquinone, reported by Morton, *et al.*, from animal organs (m.p. range 33-41°)[5] and yeast (yellow oily fractions)[6] is clearly related to the coenzyme Q group.

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MERCK, SHARP & DOHME
RESEARCH LABORATORIES
DIVISION OF MERCK & CO., INC.
RAHWAY, NEW JERSEY

DONALD E. WOLF
CARL H. HOFFMAN
NELSON R. TRENNER
BYRON H. ARISON
CLIFFORD H. SHUNK
BRUCE O. LINN
JAMES F. MCPHERSON
KARL FOLKERS

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