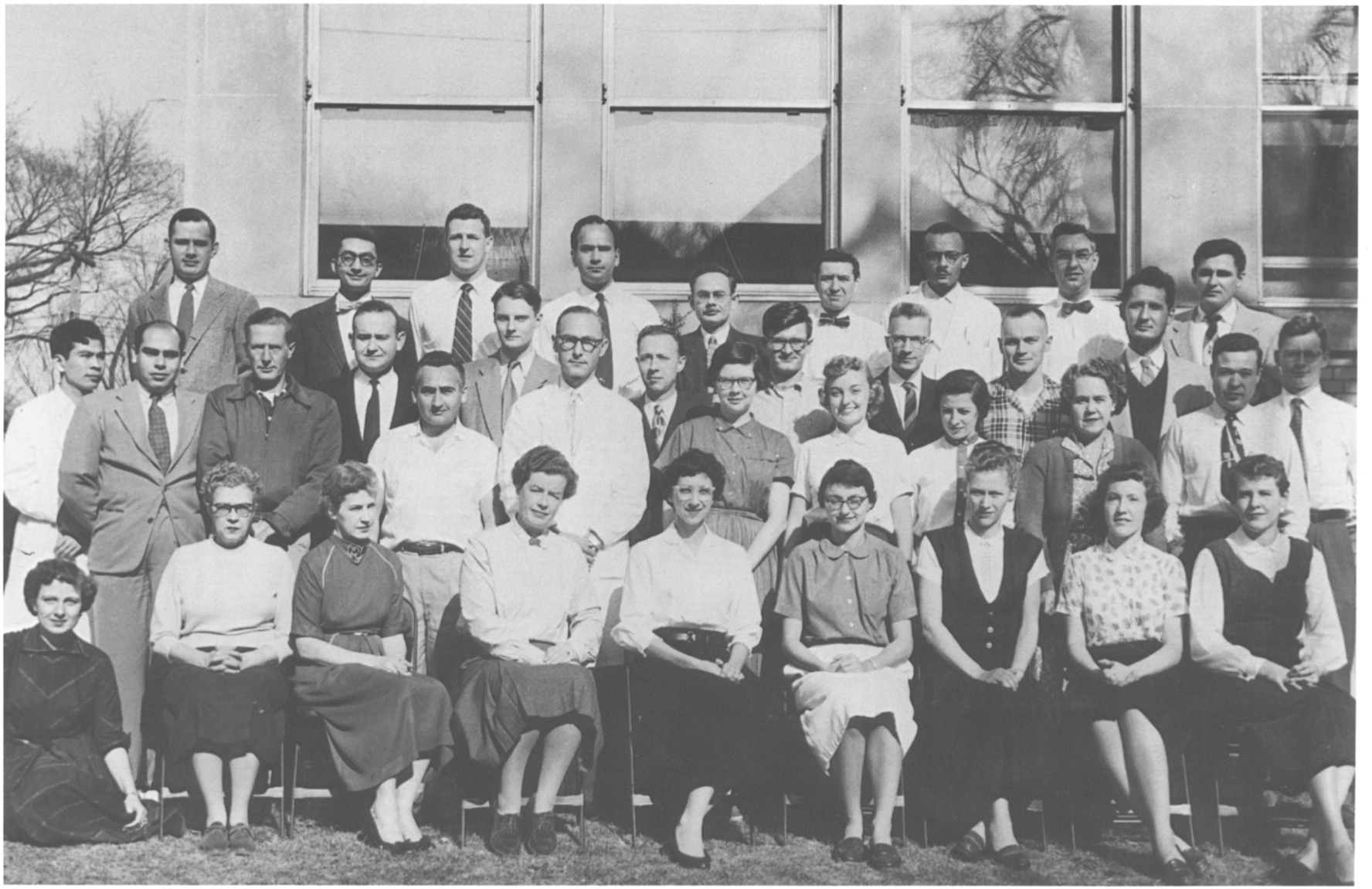
Biochimica et Biophysica Acta, 1000 (1989) 358–361
Elsevier



The staff in Dr. David E. Green's section of the Enzyme Institute, University of Wisconsin, in 1956 when coenzyme Q was discovered. Names are listed from left to right in each row.
Front row: Ruth Reitan; Amine Kalhagen; Cleo Whitcher; Elizabeth Steyn-Parvé; Jean Karr; Joanne Gilbert; Mildred Van De Bogart; Mary Benowitz; Irene Wiersma.
Second row: Seishi Kuwabara; Salih Wakil; Helmut Beinert; Alton Frost; David Green; Elizabeth Welch; Wanda Fechner; ——— Sedate Holland; Robert Labbe.
Third row: Bob Lester; Johan Jarnefelt; John Porter; ———; Bob Basford; Fred Crane; Carl Widmer; Edward Titchener (Brad).
Back row: Dave Gibson; Joe Hatefi; Tony Linnane; Dexter Goldman; Nat Penn; Bruce Mackler; Howard Tisdale; Al Heindel; Dan Ziegler.

Comments on the discovery of coenzyme Q: a commentary by

Frederick L. Crane

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on 'Isolation of a quinone from beef heart mitochondria'
by F.L. Crane, Y. Hatefi, R.L. Lester and C. Widmer
Biochim. Biophys. Acta 25 (1957) 220–221

The discovery of a quinone, subsequently named coenzyme Q or ubiquinone, in beef heart mitochondria was facilitated by the dynamic scientific atmosphere in Prof. David Green's section of the Enzyme Institute at the University of Wisconsin in 1956. Prof. Green was developing a program to take apart the mitochondrial electron transport system, to discover its working parts, and to find how this system was coupled to ATP synthesis.

We had been engaged in fractionation of the inner mitochondrial membrane with various detergents which led to the isolation of complexes of flavins and cytochromes which could carry out NADH or succinate cytochrome *c* reductase or reduced cytochrome *c* oxidase activity [1–3]. Further fractionation to separate dehydrogenases from cytochromes led to isolation of dehydrogenases which could not be reconstituted with other fractions to restore overall electron transport. It seemed as if something was missing. We had been thinking about the role of lipid, certainly as a structural element, for some time. We had studied phospholipase effects on electron transport and were analyzing the phospholipid content of the various fractions, but this did not supply an electron carrier. As a new approach, on October 26, 1956, I sent 12 g of beef heart mitochondrial protein to the Wisconsin Research Foundation Laboratories for vitamin analysis. Among the vitamins present was α-tocopherol. They did not do vitamin A analysis. During weekends I had been preparing cauliflower mitochondria, and their yellow color suggested the presence of carotenoids. Analysis of beef heart mitochondria extracts also showed the presence of carotenoids, so I embarked on an examination of carotenoids and vitamin A in heart mitochondria as an approach to possible new redox carriers. David Green was always eager for new ideas and encouraged new approaches to a problem, so he was interested in these studies of unusual lipids. It soon became apparent that the mitochondria had no detectable vitamin A, but did have a yellow compound which eluted from columns after the carotenes. This compound, prepared on December 3, 1956, had a broad absorption band around 400 nm in the visible and a very strong band at 275 nm in the ultraviolet. It did not show any Carr-Price reaction for polyconjugated double bonds, so I considered other possible chromophoric groups as a basis for the yellow color. The yellow color and absorption at 275 nm were lost upon reduction, with formation of an absorption band at 295 nm. This would be consistent with a quinone. A quinone structure was supported by R.A. Morton and his group when they proposed the name ubiquinone for the compound they had previously studied under the designation SA [2,3]. Bob Lester and I had written to Prof. Morton previously to propose that SA was a quinone like our Q_{275}. The idea of a quinone function in electron transport had considerable appeal because of my background in plant physiology, where quinones were often discussed as alternative respiratory chain components.

Bob Lester and Joe Hatefi joined Carl Widmer and me in the further definition of this quinone and a study of possible function as an electron carrier in mitochondrial electron transport. The members of the group are shown opposite. The results are summarized in our initial note (the article reprinted here). We were fortunate in the composition of the group, since each had unique skills and experience to contribute to the problem. Subsequently, T. Ramasarma, Sidney Fleischer and Kishore Ambe made important contributions in definition of the Q_{275} and its activity in restoration of succinic oxidase activity. Elizabeth Steyn Parvé was very helpful in arranging crystallographic analysis for molecular weight and shape, which indicated an unusually long side-chain and high molecular weight [3]. When David Green returned from a tour in India in the spring of 1957, he contacted Dr. Karl Folkers, who

immediately was interested in working on the definition of this new compound because of its potential role as a vitamin. This was the beginning of a fruitful collaboration with the group at Merck Sharpe and Dohme Laboratories, who proceeded with complete elucidation of the structure and synthesis of the series of compounds, which were named coenzyme Q because of the evidence for a function in mitochondrial electron transport [3].

The solvent extraction approach which we used to demonstrate activity restoration and analyze quinone specificity was pioneered by Al Nason and co-workers [4] in their study of possible tocopherol function. We had studied this effect in mitochondrial fractions and found it was not specific for tocopherol. We went back to this approach and found it could be modified for extraction of coenzyme Q and specific restoration of succinate cytochrome *c* reductase by added coenzyme Q [3].

The initial effect of this discovery was to expand the understanding of mitochondrial electron transport. It was the key to the final success in separation of the parts of the electron transport chain and putting them back together to restore the complete system [5,6]. Coenzyme Q was the missing component needed for definition of complexes I, II and III of the mitochondrial electron transport chain. Beyond that, it helped to develop the new concept of chemiosmosis as a basis for mitochondrial ATP synthesis. In 1961, Peter Mitchell [7] incorporated coenzyme Q into his transmembrane electron transport loops. The presence of a lipid soluble electron and proton carrying quinone made the transmembrane proton movement in chemiosmosis a more logical approach to ATP synthesis.

The studies on restoration of activity with coenzyme Q also led to wider appreciation of phospholipid function in membrane-bound enzyme systems.

Our studies on coenzyme Q distribution led directly to rediscovery of plastoquinone, which had been observed by Kofler in alfalfa some years before [8]. Since plastoquinone was concentrated in chloroplasts, it was obvious that it could have an analogous redox function in photosynthetic electron transport. We found that the plastoquinone was reduced or oxidized when chloroplasts were exposed to light or dark, which was the beginning of extensive studies of plastoquinone function in photosynthesis [3,9]. The discovery of coenzyme Q in mitochondria thus led to an overall concept of lipophilic quinone function in energy coupling electron transport systems.

The significance of coenzyme Q in nutrition or medicine is still being elucidated. Dr. Karl Folkers has led studies to detect and alleviate any coenzyme Q deficiency diseases [10]. Early studies of various idiopathic diseases, e.g., muscular distrophy or kwashiorkor, did not show any conclusive effects. We began to talk about coenzyme Q as the vitamin which was so essential that a deficiency could not be tolerated. Later studies have been reported in a series of symposia organized by K. Folkers and Y. Yamamura on the biomedical and clinical aspects of coenzyme Q [11–15]. Extensive, controlled clinical studies in several medical centers have demonstrated a significant improvement in some cases of congestive heart failure following treatment with coenzyme Q. The high level of success in alleviating symptoms with long-term coenzyme Q therapy is remarkable in view of the multitude of known sites for mitochondria failure, many of which would not be expected to be relieved by coenzyme Q.

There have been repeated observations of positive effects of coenzyme Q therapy in several other conditions, including periodontal disease, immunodeficiencies and hypertension [10]. These effects have not been as carefully and extensively studied as the treatment of heart failure, so further studies are needed.

Coenzyme Q in both the oxidized and reduced form has been shown to be an excellent antioxidant for protection against degradative effects of oxygen radicals. The antioxidant action has been proposed as an approach to control of damage by antitumor drugs which produce oxygen radicals by autooxidation which damage healthy tissue [16]. This role of coenzyme Q requires more study, especially in light of evidence that the quinone is not restricted to mitochondrial membranes but is found in all endomembranes and plasma membranes [11,15]. Coenzyme Q may be the major antioxidant present in many membranes and therapeutic effects may not be limited to restoration of mitochondrial energy conversion.

I believe that the coenzyme Q story once again demonstrates that off-the-main-track research around a major problem can lead to revolutionary advances. There is another aspect which has not been commonly recognized. We were fortunate to be able to publish the material from Dr. Green's section at the Enzyme Institute in BBA in the 1950's since other biomedical journals at that time were not readily receptive to our manuscripts. In this paper the receptiveness to new ideas at BBA under Editor H.G.K. Westenbrink has stood the test of time.

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Isolation of a quinone from beef heart mitochondria*

From lipid extracts of beef heart mitochondria we have isolated a new compound capable of undergoing reversible oxidation and reduction. The absorption spectrum of the oxidized and reduced forms are shown in Fig. 1. The oxidation-reduction behaviour as well as the infrared spectrum indicate that the compound is a quinone. For convenience it will be referred to as Q-275. A compound with similar spectral properties has been observed also in beef liver mitochondria. This yellow-orange, crystalline material has been recrystallized from several solvents to a constant melting point (48–49° C) and to a constant extinction coefficient. The purity of the compound is being investigated by chromatographic procedures. Q-275 is insoluble in water, but is soluble in most lipid solvents.

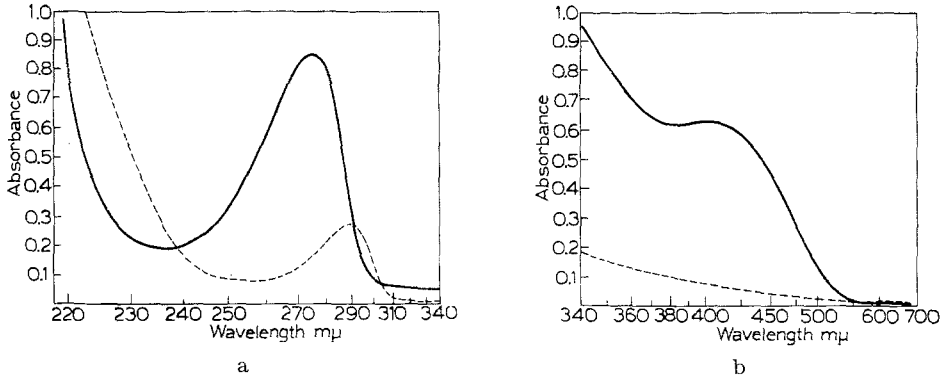

Fig. 1. Absorption spectrum of Q-275 in absolute ethanol. Solid line represents oxidized form, and the dotted line represents the spectrum obtained after shaking with a few grains of KBH_4.
Concentrations used for 1 cm path in mg/ml: Ultraviolet range, 0.0425; visible range, 0.75.

In addition to beef heart mitochondria, Q-275 has been found in various electron transporting particles derived from these mitochondria. The concentration of Q-275 (mg/g protein) was found to be as follows: Mitochondria[1], 2.5; ETP[1], 2.7; SDC[2], 6.0; green fraction[3], 0.5. The presence of Q-275 appears to be correlated with succinate oxidizing capacity.

That Q-275 is involved in the electron transport activities of the aforementioned particles is indicated by several lines of evidence.

* This work was supported in part by a training grant (No. HTS-5006) and a research grant (No. H-458) from the National Heart Institute, Public Health Service. The tissues were kindly supplied by Oscar Mayer and Co.

I. After exposure of mitochondria to air, the extracted compound is observed to be in the oxidized form. After a short incubation in the presence of succinate and cyanide, the compound then appears in the reduced form. This reduction is inhibited by Antimycin A. The same effect cannot be duplicated by similar incubation of the particles with DPNH.

II. It has been possible to show that the oxidation and reduction of externally added Q-275 is catalyzed by mitochondria and derivative particles. Reduction of added Q-275 by succinate occurs in the presence of mitochondria, ETP and SDC; by DPNH in presence of mitochondria and ETP; and by pyruvate plus malate in presence of mitochondria. The reduction occurs only in the presence of cyanide or under anaerobic conditions. The oxidation of the chemically reduced compound is catalyzed by mitochondria or ETP. This oxidation is inhibited by cyanide. The reduced compound is oxidized by ferricytochrome *c* in the presence of ETP or SDC.

III. When mitochondria or ETP are extracted with heptane or 2,2,4-trimethylpentane, Q-275 appears in the organic solvent. This extraction decreases the succinoxidase activity; addition of Q-275 restores the initial activity. Although no detectable cytochrome *c* is released from the particles by this treatment, addition of cytochrome *c** also restores activity (Table I). The restored succinoxidase system is completely inhibited by antimycin A at 0.002 mg/1.68 mg protein. Other compounds which were tested as substitutes for Q-275 either had no effect or inhibited the low blank rate. The compounds** tested which had no effect were *p*-xyloquinone; menadione; 2-hydroxy-1,4-naphthoquinone; 3-methyl-1,2-naphthoquinone; bovine serum albumin; *d*-α-tocopherol; vitamin K_1; β-carotene; 2,3-dimethyl-1,4-naphthoquinone, 2-undecyl-1,4-naphthoquinone; and tuna fish oil. The blank rate was inhibited by lapachol, norlapachol and norlapachol acetate. All these compounds were added at concentrations comparable to that of Q-275.

TABLE I

EFFECT OF Q-275 ON SUCCINOXIDASE

Enzyme treatment	*Additions*	*Activity μatoms oxygen/5 min/mg*
None	—	2.5
None	Q-275, 0.088 mg	2.5
None	cytochrome *c*, 1 mg	2.6
Extracted	—	0.4
Extracted	Q-275, 0.088 mg	2.7
Extracted	cytochrome *c*, 1 mg	2.9

Assay for succinoxidase at 38° as previously reported[1] except air used as gas phase. 2.5 mg of ETP, and 1.58 mg of extracted ETP used in a total volume of 1.5 ml. Q-275 added in 0.03 ml ethanol.

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Received April 18th, 1957

* Purchased from Sigma Biochemical Co.

** We wish to thank Professor L. F. FIESER of Harvard University and Professor H. BEINERT of this laboratory for kindly supplying samples of the quinones tested.

*** Postdoctoral Trainee of the National Heart Institute, National Institutes of Health.