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# Discovery of ubiquinone (coenzyme Q) and an overview of function

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**Abstract**

Details of the discovery of ubiquinone (coenzyme Q) are described in the context of research on mitochondria in the early 1950s. The importance of the research environment created by David E. Green to the recognition of the compound and its role in mitochondria is emphasized as well as the dedicated work of Karl Folkers to find the medical and nutritional significance. The development of diverse functions of the quinone from electron carrier and proton carrier in mitochondria to proton transport in other membranes and uncoupling protein control as well as antioxidant and prooxidant functions is introduced. The successful application in medicine points the way for future development.


*Keywords:* Bioenergetics; Uncoupling protein; Antioxidant; Oxygen radical; Therapeutic; Nutrition

The discovery of coenzyme Q was not a simple accident as sometimes mentioned. It was the result of a long train of investigation into the mechanism of, and compounds involved in biological energy conversion. Its origin can be traced back to the early studies of biological oxidation especially those of Warburg and Keilin which introduced a chain of cytochromes as electron carriers to oxygen. These studies were extended by Chance (1954) with the development of the rapid flow-dual beam spectrophotometer. With this instrument the rate of reduction of the component flavins and cytochromes could be measured which led to definition of an electron transport chain from NADH or succinate through flavin, cytochrome *b*, cytochrome *a*, cytochrome *c*, cytochrome *a* and $a_3$ to oxygen.

The next question was how these cytochromes were associated and how they drove the formation of ATP.

In the early 1950s good biochemistry required the purification of enzymes for proper definition of their catalytic properties. When David Green proposed that the citric cycle and oxidative phosphorylation was contained in an organized complex which he called cyclophorase it was met by extreme skepticism (Green et al., 1948). The development of microscopic staining and ultracentrifuge techniques to identify the respiratory particles as mitochondria has been reviewed by Lehninger (1964). In 1950 Green at the Enzyme Institute, University of Wisconsin then embarked on a major program to determine how the enzymes of the fatty acid oxidation and citric cycle oxidation were organized and how this contributed to energy coupling in oxidative phosphorylation. A unique and crucial component of this program was isolation of large amounts of mitochondria. Most laboratories involved in these studies used small amounts of rat liver or pigeon breast muscle mitochondria which gave good ratios of ATP formed to oxygen consumed (P/O ratios) approaching 3 but left little to work with in enzyme isolation.

To obtain a large amount of material to work with Green arranged to get up to a dozen beef hearts per day from the Oscar Meyer plant in Madison. These hearts were homogenized in a large blender followed by centrifugation in a large 13 l centrifuge to remove unwanted material such as myosin. The supernatant was then centrifuged in a large industrial size sharples machine to sediment the mitochondria as a brown paste which were suspended in a sucrose phosphate buffer prior to freezing. The production was

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80–100 g of mitochondrial protein per day. To start a study we could just go to the freezer and take out a liter of concentrated mitochondria.

With a good supply of mitochondria available Green instituted a program to systematically separate parts of the electron transport system to see how they interacted and how this interaction was related to ATP formation. By using aqueous extractions it was possible to prepare isolated flavoproteins, succinc dehydrogenase and NADH dehydrogenase, which could react with artificial electron acceptors but not with cytochrome *c*. Further fractionation required the use of detergents. The first success was separation of a succinate cytochrome *c* reductase preparation which contained flavin, cytochrome *b* and cytochrome *c*1 by Green and Burkhard (1961). In the meantime Wainio et al. (1948) using deoxycholate had succeeded in separating a cytochrome *c* oxidase which contained only cytochromes *a* and $a_3$. This led to studies with cholate detergents which gave other fractions most notably a NADH cytochrome *c* reductase. Further fractionation led to loss of activity. Until the discovery of coenzyme Q made it possible for Hatefi et al. (1962) to reconstruct the complete electron transport system. During these fractionation studies it became clear that the lipid in the membrane was closely associated with the cytochromes and flavine. According to theories of membrane structure at that time (late 1950s) the components of the electron transport would be bound to the surfaces of the mitochondrial lipid bilayer. Since the products of fractionation all retained lipid it became apparent that the association of the carrier protein and lipid was very strong. This led to a consideration of the possible functional role of lipid. At this point I felt that detergent based fractionation of the mitochondrial membranes had been exploited as much as possible so Carl Widmer and I started a study of the lipids associated with the various fractions. At about this time (1955) Nason and coworkers (Donaldson et al., 1958) reported inducing a requirement for α-tocopherol in mitochondrial electron transport by isooctane extraction. I tried the effect of isooctane on mitochondrial electron transport and found isooctane induced inhibition could be reversed by α-tocopherol but that the reversal could also be achieved with beef serum albumin. I wrote to Nason and he agreed that serum albumin had something that reversed the isooctane inhibition. These experiments led to two considerations: first that the electron transport proteins were remarkably resistant to denaturation by hydrocarbon solvents which laid the ground work for the use of these solvents to extract coenzyme Q in studies of its activity. Second it led me to consider if any other vitamin might be needed in the electron transport, loss of which might account for fractions where activity could not be restored. To see what vitamins might be involved I sent a liter of mitochondria to the Wisconsin Alumni Research Laboratory for vitamin analysis. Several B vitamins were found in the beef heart mitochondria preparation, but no niacin. A significant amount of α-tocopherol was present. In separate assays I found no vitamin K. The research laboratory did not do vitamin A analysis so I set out to do that. Because of my plant physiology background I was interested in plant mitochondria. Since the Enzyme Institute was uniquely equipped for study of mitochondria I took advantage of the facilities to make cauliflower mitochondria on the weekends when the laboratory was essentially empty (Crane, 1957). Surprisingly the cauliflower mitochondria were yellow, not brown like animal mitochondria. So the thought occurred to me that the cauliflower mitochondria might contain carotenoids and that beef heart mitochondria might have carotenoids hidden under the brown pigments. I figured that many compounds with absorption spectra in the visible range had loose electrons and could act as electron carriers in oxidation reduction reactions so carotenoids were possible carriers. It turned out that beef heart mitochondria have carotenoids but no vitamin A. If the amount of carotenoids is estimated as β carotene by spectral absorption of unsaponifiable lipid at 448 nm the 9 nmole/mg protein found is higher than the concentration of cytochromes (0.8–0.4 nmole/mg protein) so it would be sufficient to function in electron transport. The lack of vitamin A was confirmed by a negative Carr–Price reaction. When the non-saponifiable lipid was chromatographed on an alumina or Decalso column three carotenoid bands were eluted with heptane. Following the carotenoids was a broad yellow band which eluted off the column with 2% ethyl ether in heptane. This compound had a broad absorption peak around 400 nm and was first observed December 3, 1956. Later it was found to have a strong peak at 275 nm. To guard against saponification artifacts we prepared the "400" compound from a petroleum ether–ethanol extract of mitochondria. Removal of the solvent from the "400" fraction eluate left a yellow oil. When the oil was dissolved in ethanol and put in the refrigerator for 2 days long needle like yellow crystals formed which could be filtered off. These were recrystalized until a constant melting point of 48–49 °C was maintained (Crane et al., 1957). The next question was what was this strange compound and could it have a role in electron transport? We knew that it was probably not a short chain carotenoid (like colorless vitamin A) or a long chain carotenoid (like β carotene) because of lack of multiple peaks in the visible spectra (400 nm) and lack of Carr Price reaction (40 carbon poly unsaturated carotenes like β carotene show Carr Price peaks in the infrared region ~ 1000 nm). In the organic chemistry course at the University of Michigan Prof Bachman taught organic chromophore which included quinones. As a graduate student in plant physiology I was exposed to discussion of possible roles of quinones in plant respiration which was not expected in animals because benzoquinones were reported to be rare in animals (Thomson, 1957). Bob Lester and I discussed the idea that the 400 compound might be a quinone. We dug a small book by Morton on organic spectra out of the library and found the spectrum of benzoquinone with an oxidized peak at 254 nm and a smaller peak in the