

## A touch of history and a peep into the future of the lipid-quinone known as coenzyme Q and ubiquinone

T. Ramasarma

*Coenzyme Q (ubiquinone), a fully substituted benzoquinone with polyprenyl side chain, participates in many cellular redox activities. Paradoxically it was discovered only in 1957, albeit being ubiquitous. It required a person, F. L. Crane, a place, Enzyme Institute, Madison, USA, and a time when D. E. Green was directing vigorous research on mitochondria. Located at the transition of 2-electron flavoproteins and 1-electron cytochrome carriers, it facilitates electron transfer through the elegant Q-cycle in mitochondria to reduce O<sub>2</sub> to H<sub>2</sub>O, and to H<sub>2</sub>O<sub>2</sub>, now a significant signal-transducing agent, as a minor activity in shunt pathway (animals) and alternative oxidase (plants). The ability to form Q-radical by losing an electron and a proton was ingeniously used by Mitchell to explain the formation of the proton gradient, considered the core of energy transduction, and also in acidification in vacuoles. Known to be a mobile membrane constituent (microsomes, plasma membrane and Golgi apparatus), allowing it to reach multiple sites, coenzyme Q is expected to have other activities. Coenzyme Q protects circulating lipoproteins being a better lipid antioxidant than even vitamin E. Binding to proteins such as QPS, QPN, QPC and uncoupling protein in mitochondria, QA and QB in the reaction centre in R. sphaeroides, and disulfide bond-forming protein in E. coli (possibly also in Golgi), coenzyme Q acquires selective functions. A characteristic of orally dosed coenzyme Q is its exclusive absorption into the liver, but not the other tissues. This enrichment of Q is accompanied by significant decrease of blood pressure and of serum cholesterol. Inhibition of formation of mevalonate, the common precursor in the branched isoprene pathway, by the minor product, coenzyme Q, decreases the major product, cholesterol. Relaxation of contracted arterial smooth muscle by a side-chain truncated product of coenzyme Q explains its effect of decreasing blood pressure. Extensive clinical studies carried out on oral supplements of coenzyme Q, initially by K. Folkers and Y. Yamamura and followed many others, revealed a large number of beneficial effects, significantly in cardiovascular diseases. Such a variety of effects by this lipid quinone cannot depend on redox activity alone. The fat-soluble vitamins (A, D, E and K) that bear structural relationship with coenzyme Q are known to be active in their polar forms. A vignette of modified forms of coenzyme Q taking active role in its multiple effects is emerging.*

‘An ounce of creativity is worth a ton of impact’ – Joseph Goldstein

Coenzyme Q, also known as ubiquinone, was discovered in 1957 with inputs from a university (Wisconsin, USA and Liverpool, UK) and a pharmaceutical company (Merck, Sharpe & Dohme, USA and Hoffman-La Roche, Switzerland), each on either side of the Atlantic Ocean. The 5th Conference of the International Coenzyme Q<sub>10</sub> Association held in 2007 in Kobe University, Japan commemorated 50 years of this discovery. The conference organizer, T. Kishi, programmed my talk on the historical perspective with a glimpse into the future in the inaugural session after the first talk by the discoverer, Frederick L. Crane. This article is an extended version of that presentation with added attention on potential functions of Q-binding proteins and Q-derived metabolites.

### Discovery of the lipid-quinone, named coenzyme Q and ubiquinone

Why did it take so long to discover coenzyme Q? Ubiquitous in animals, plants and many microorganisms, it could have been found anywhere in the world in any of the many laboratories working on natural products. A discovery needs a person, a place and a time to come together. F. L. Crane isolated a golden-yellow crystalline compound from lipids of beef heart mitochondria, abundantly available in the Enzyme Institute in the University of Wisconsin, Madison, USA, where D. E. Green was directing an intensive study of mitochondrial electron transfer chain at that time. Crane, Robert Lester and Yousef Hatefi (Figure 1), found that this yellow compound could be reversibly reduced with loss of the colour, its  $\lambda_{\max}$  shifting from 275 to

290 nm. This finding led to the identification of a quinone as chromophore of the compound, referred to as Q275. The first note on the discovery of coenzyme Q by Crane *et al.*<sup>1</sup> was published in 1957 in *Biochimica Biophysica Acta*, a popular international journal with no extraordinary impact factor (Figure 1). The modest claim of ‘isolation of a quinone from beef heart mitochondria’ with redox properties would have remained unnoticed in the absence of the glamour associated with the work on bioenergetics at that time before the advent of molecular biology. The name coenzyme Q suggested itself after its coenzyme role in the mitochondrial electron transport chain was established. In an eminently readable article, Crane<sup>2</sup> gave an account of the initial efforts leading to the discovery and an overview of the functions of coenzyme Q that emerged in the following five decades. The year 1957 also

## HISTORICAL NOTES

coincided with the publication of the book by R. H. Thomson claimed to be a comprehensive account of naturally occurring quinones. Yet to be discovered, coenzyme Q (ubiquinone) was conspicuous by its absence (Figure 1).

A necessary factor for creative work is the ambience provided by the laboratory, the leader and the co-workers. And this was abundant in the Enzyme Institute during that period. Acetyl CoA carboxylase, the first enzyme in fatty acid synthesis, was also discovered in that year by Salih Wakil. I was fortunate to be part of that vigorous team during 1957–1958 and have continued our studies on this newly discovered compound ever since. I enjoyed the work and the monthly social meetings at homes of the international team assembled by David Green.

About the same time in the late 1950s, the Liverpool group led by R. A. Morton<sup>3</sup> found a compound, referred to as SA, with  $\lambda_{\text{max}}$  275 nm accumulated in lipids of livers of animals deficient in vitamin A. Exchange of information between the two laboratories led to agreement that SA and Q275 are the same. The Liverpool group named it ubiquinone because of its ubiquitous occurrence<sup>4</sup>. Morton

shared with us at the meeting in Bangalore (Figure 2) in 1976 that according to the Vice-Chancellor of the Liverpool University, the proper English word is 'ubiquinone' and this jaw-jerker 'quiqui' mercifully never came to light. Both names coenzyme Q and ubiquinone coexist in literature without conflict, often in the same paper.

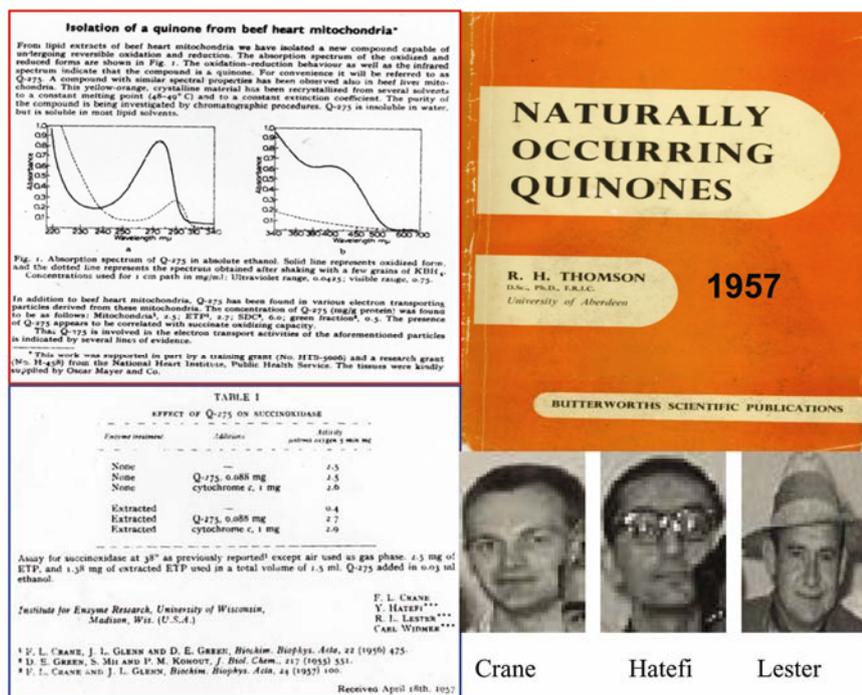
As early as 1940, Thomas Moore and Krishna Rao Rajagopal (of Indian origin) at the University of Cambridge, UK identified an alkali-sensitive compound with  $\lambda_{\text{max}}$  275 nm that extracted with vitamin E, but without a clue on its identity<sup>5</sup>. I could not trace Rajagopal but obtained a group photograph of Moore (inset, Figure 2) taken along with Morton, A. T. Diplock and R. E. Olson, also coenzyme Q workers, at a meeting organized by H. R. Cama and P. S. Sastry held in Indian Institute of Science (IISc), Bangalore.

The structure of the compound, 2,3-dimethoxy 5-methyl 6-decaprenyl 1,4-benzoquinone (Q<sub>10</sub>; see below), was solved in 1958 by the groups at Merck<sup>6</sup> and Hoffman-La Roche<sup>7</sup> with amazing rapidity, what with the new methods of structure determination available by that

time. Also in 1958, Gloor and Wiss<sup>8</sup> found that mevalonate-2-<sup>14</sup>C, the biological precursor of isoprene units, was incorporated into ubiquinone indicating its biosynthesis in animal tissues. This dampened any consideration of vitamin status for ubiquinone (Scheme 1).

Hatefi announced the arrival of coenzyme Q in biochemistry at the International Union of Biochemistry conference in Vienna in 1958. I hardly noticed at that meeting the vibration expected regarding important discovery that it turned out to be. It fits with the 'top-down' type of discoveries described by Goldstein<sup>9</sup>, whose 'impact becomes ever larger' with the passage of time 'before their full biological and medical importance is appreciated'.

It is appropriate to salute the pioneers who made possible the discovery, characterization, and identification of the potential health benefits of coenzyme Q: David Green (Wisconsin University, USA), Karl Folkers (Merck, Sharpe & Dohme, USA), Richard Morton (Liverpool University, UK) and Otto Isler (Hoffman-La Roche, Switzerland), and Yuichi Yamamura (Japan; Figure 3).



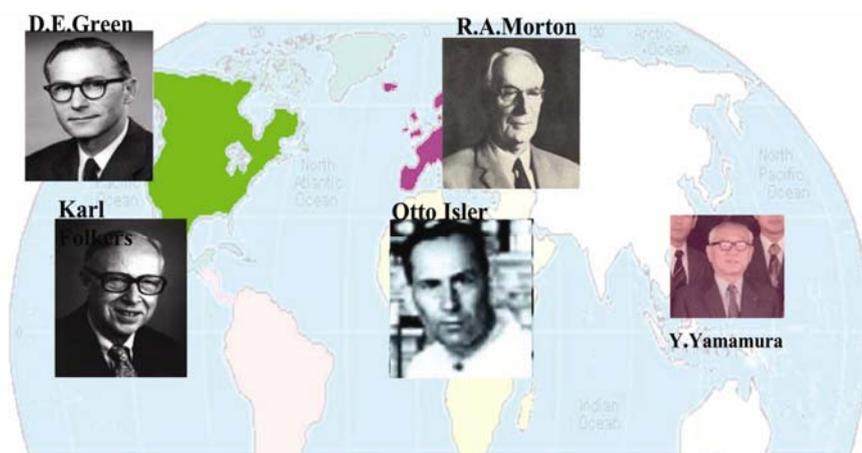
**Figure 1.** The first note on the discovery of coenzyme Q published in *Biochimica Biophysica Acta*<sup>1</sup>. Called Q275 then, its oxidized and reduced spectra and the effect on succinoxidase was reported. Insert at the right bottom shows the photographs of Crane, Hatefi and Lester in 1957. The book of Thomson on *Naturally Occurring Quinones* appeared in the same year with no mention of the just discovered ubiquinone.

## Coenzyme Q fills the slot of mobile lipid electron carrier

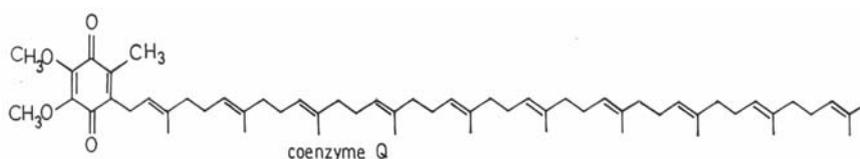
Emergence of coenzyme Q illustrates the importance of association of function of a new compound after isolation and chemical identification. With the expertise available at the Enzyme Institute, the redox function of coenzyme Q in the electron transport system of mitochondrial inner membrane was quickly identified and it was assigned the role of a lipid-member linking flavoprotein dehydrogenases and cytochrome chain<sup>10</sup>. The electron transport chain known at that time (A, Table 1) was expanded to include coenzyme Q (B, Table 1). The lipid-soluble coenzyme Q distributed in the membrane, and the water-soluble cytochrome *c* loosely attached to the membrane, form an elegant pair of detachable components that effectively control the electron flow through to oxygen. After the discovery<sup>11</sup> that the flavoproteins and cytochromes are organized as transmembrane complexes I–IV (ref. 11), coenzyme Q and cytochrome *c* acting as bridges between them (C, Table 1) became self-evident.



**Figure 2.** Group photographs of participants at the Symposium on 'Vitamin and Carrier Functions of Polyprenoids' organized by H.R. Cama and P.S. Sastry, in front of the founder's statue at the Indian Institute of Science, Bangalore, India in December 1975. Thomas Moore is identified by the red oval and shown in the inset. Other coenzyme Q workers, A. T. Diplock, R. A. Morton, A. S. Aiyar and R. E. Olson are also seen in the first row.



**Figure 3.** Pioneers who made possible the discovery of coenzyme Q/ubiquinone, and understanding its role in biological oxidations and its biomedical applications: D. E. Green (University of Wisconsin, USA) and Karl Folkers (Merck, Sharpe & Dohme, USA), R. A. Morton (University of Liverpool, UK) and Otto Isler (Hoffman La Roche, Switzerland), Y. Yamamura (Japan) and Folkers initiated clinical trials on coenzyme Q that revealed its cardiovascular benefits and biomedical value.

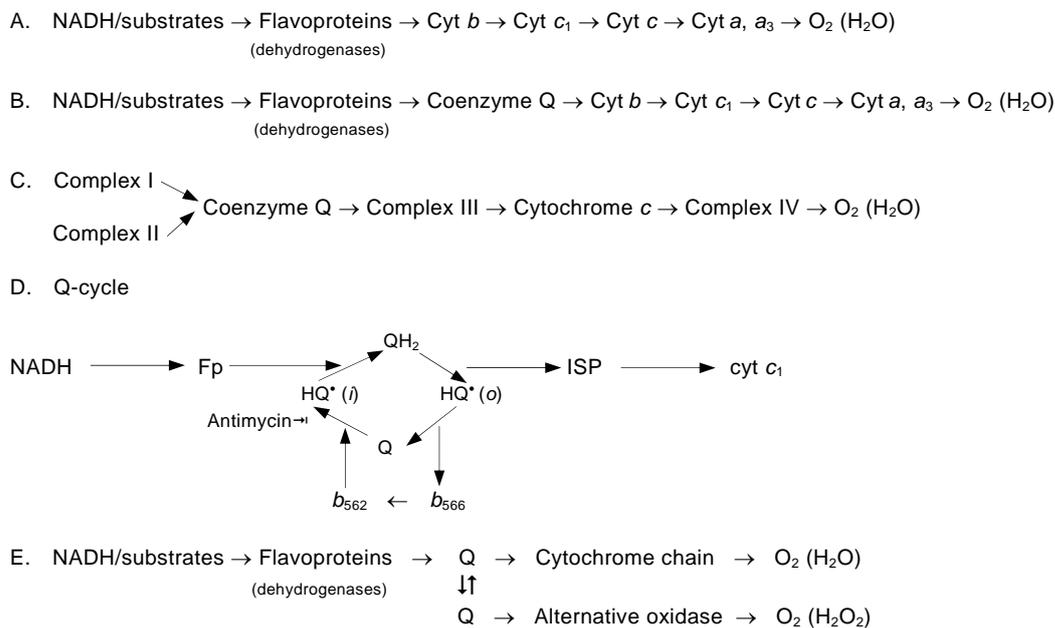


**Scheme 1.**

Inclusion of coenzyme Q in mitochondrial electron transfer sorted out another nagging feature of transition of two-electron systems up to flavoproteins (Fp) into one-electron cytochrome chain. The semiquinone (one electron depleted quinone,  $HQ^{\bullet}$  radical) mediates in electron transfer through the Q-cycle<sup>12</sup> – it receives an electron from NADH through flavoprotein (Fp) dehydrogenases on one side (*i*) of the membrane and donates it to Fe-sulphur protein (ISP)/cytochrome  $c_1$  on the other side (*o*). The cyclic operation includes normalizing the electron through cytochromes  $b_{566}$  and  $b_{562}$  to regenerate semiquinone on the other side of the membrane (D, Table 1). The same could have been achieved using the redox couple of  $QH_2 - HQ^{\bullet}$  or  $HQ^{\bullet} - Q$ , but without translocating protons across the membrane. Mitchell<sup>13</sup> ingeniously used the Q-cycle and its vectorial transfer of protons for creating a proton gradient to explain his chemiosmotic theory of energy transduction to ATP. This indeed made coenzyme Q famous for its unique role in coupling transfer of electrons, protons and energy, and became publicity flash to the molecule as one that supports cellular energy production.

## HISTORICAL NOTES

**Table 1.** The placement of coenzyme Q in the mitochondrial electron transport chain. Coenzyme Q acts as a mobile electron transport component linking two-electron transfer flavoproteins and one-electron transfer cytochromes and transfers electrons through the Q-cycle



The product of reduction of oxygen is shown in parenthesis.

### H<sub>2</sub>O<sub>2</sub> generation dependent on coenzyme Q

A varying proportion of oxygen consumed by mitochondria of different tissues (about 1% in animals) or alternative oxidase (AOX; up to 50% in plants) is insensitive to cyanide, and therefore not dependent on cytochrome oxidase. This activity is sensitive to phenolic acids in animals<sup>14</sup> and salicylhydroxamate (SHAM) in plants. This shunt pathway diverts electrons from the Q-pool by autoxidizable proteins to produce H<sub>2</sub>O<sub>2</sub> as the reduction product of O<sub>2</sub> (E, Table 1). In 1976, Boveris *et al.*<sup>15</sup> measured the small rates of H<sub>2</sub>O<sub>2</sub> generated in the mitochondria and demonstrated the expected involvement of ubiquinone. At about the same time, Storey<sup>16</sup> had confirmed the participation of ubiquinone in plant AOX activity, and Rich *et al.*<sup>17</sup> obtained dependable evidence for H<sub>2</sub>O<sub>2</sub> as its product of reduction of O<sub>2</sub>, overcoming difficulties with H<sub>2</sub>O<sub>2</sub>-degrading enzymes. The work of Radha Bhat (Agharkar Institute, Pune) in 2009 with chilled potato mitochondria reaffirmed this<sup>18</sup>, by showing that H<sub>2</sub>O<sub>2</sub> is the product of SHAM-sensitive and cyanide-insensitive reduction of O<sub>2</sub> by AOX by the release of half of the consumed oxy-

gen into the medium on adding excess catalase, an incontrovertible evidence for presence of H<sub>2</sub>O<sub>2</sub>. Alerting the need for correcting the intervening unsupported assertions on H<sub>2</sub>O (water) as the product (see Møller *et al.*<sup>19</sup>), and explaining the mistaken basis<sup>20</sup> of this incorrect inference, we made a plea for reinstating H<sub>2</sub>O<sub>2</sub> as the product of reduction of O<sub>2</sub> by AOX in plants<sup>21</sup>. This hopefully brings back into focus the physiological role of H<sub>2</sub>O<sub>2</sub>, and also of Q, in AOX.

Cyanide-insensitive respiration and AOX activity are not limited to plants. Working in our laboratory, Kaleysa Raj (Kerala University, Thiruvananthapuram) found that a vigorously motile filarial parasite (*Setaria digitata*) from the peritoneal cavity of cattle, survived in presence of 30 mM KCN. Mitochondria prepared from these worms contained several dehydrogenases and coenzyme Q, but no cytochromes, and also the activity of H<sub>2</sub>O<sub>2</sub>-generating cyanide-insensitive respiration<sup>22</sup>. H<sub>2</sub>O<sub>2</sub>, known to contract smooth muscle, appears to be the energy source for the wriggling movements of these parasites. Extensive studies by Raj and his co-workers<sup>23</sup> in his laboratory on the unusual oxidative metabolism of this parasite showed the presence of two homologues of coenzyme Q, of which

only Q<sub>6</sub>, present in the adult microfilarial stage, is redox active and produces H<sub>2</sub>O<sub>2</sub>, whereas Q<sub>8</sub> is supposed to have antioxidant function<sup>23</sup>. The homologue, Q<sub>8</sub>, may be derived from bacteria that the worms feed on, and is known to be enriched in excretory–secretory material the worms throw out.

The occurrence of twin homologues of coenzyme Q is known in another worm, *Caenorhabditis elegans*, which had native Q<sub>9</sub> and also Q<sub>8</sub> derived from *Escherichia coli* which they feed on. In 2002 Clarke and colleagues<sup>24</sup> found that *clk-1* mutant of this worm characteristically accumulated demethoxy-Q<sub>9</sub>, the non-functional precursor of Q<sub>9</sub>. When dietary supplement of Q<sub>8</sub> was withheld, these worms exhibited ‘either arrest of development as young larvae or become sterile adults’ underlining the importance of coenzyme Q in growth and development. Their work<sup>25</sup> on the extension of the lifespan of the worms in the absence of Q<sub>8</sub> in the diet supplement led to linking reactive oxygen species and insulin receptor action with the phenomenon of senescence of these worms. Increased H<sub>2</sub>O<sub>2</sub> generation by the exogenous Q<sub>8</sub> under these conditions is a possible cause. H<sub>2</sub>O<sub>2</sub> is known to accelerate senescence, typically shown recently in

fibroblasts by Gayatri and colleagues<sup>26</sup> (CDFD, Hyderabad), and is distinguished as 'a common mediator of ageing signals' according to Giorgio *et al.*<sup>27</sup>.

### Binding of coenzyme Q to specific proteins

From the very early studies<sup>28</sup> in the Enzyme Institute, part of mitochondrial coenzyme Q was known to resist reduction and this appears to be the free lipid-quinone in the membrane bilayer, reached by absorption of exogenous form or added to isolated mitochondria. In 1984, Gupte *et al.*<sup>29</sup> documented mobility of Q molecules in the membrane that are often represented as mobile electron carrier along with cytochrome *c* in the electron transport chain. But only protein-bound lipid-quinone seems to interact with other electron-transfer proteins and becomes redox-active. Three of these proteins are obtained by fractionation of mitochondrial membrane protein complexes with multiple molecules of Q-bound and differing molecular masses: QP-N in complex I (14.0 kDa), QP-S in complex II (13.0 kDa, 15.5 kDa), and QP-C in complex III (15.0 kDa, 12.5 kDa) from the work of King<sup>30</sup>, Yu *et al.*<sup>31</sup>, Suzuki and Ozawa<sup>32</sup>. Possessing small differences of redox potential because of binding to different proteins and the ability to move in the membrane, their redox operation rationalized the paradox of HQ• radical giving an electron to cytochrome *b*<sub>562</sub> and Q receiving it back from cytochrome *b*<sub>566</sub> in the Q-cycle (E, Table 1). These Q-bound proteins facilitate electron transfer to acceptors and to Q, and also stabilize the HQ• radicals. Binding of coenzyme Q to proteins is a natural property of the lipid-quinone. Thus it also gains multiple activities. This is exemplified by the acceptor quinones – Q<sub>A</sub> and Q<sub>B</sub> – in the photosynthetic reaction centre of *Rhodobacter sphaeroides*, a purple photosynthetic bacterium, where such binding fine-tunes the redox potential<sup>33</sup>. The 2-methoxy group of coenzyme Q is essential for this activity. For the first time the methoxy group, unique to coenzyme Q and not present in other lipid-quinones, is implicated in a functional role as a facilitator of electron exchange in reversible formation of HQ• radical.

One other example of coenzyme Q bound to a protein is the mitochondrial

uncoupling protein (UCP). This protein occurs in large amounts in mitochondria of brown adipose, a thermogenic tissue. Klingenberg and coworkers<sup>34</sup> found that coenzyme Q was present in the isolated protein and the bound Q acted as a cofactor in fatty acid-dependent transport of H<sup>+</sup> across the membrane instead of through ATP-synthase complex V, thus releasing energy as heat.

Oxidation of protein thiols to disulphide and reduction of oxygen to H<sub>2</sub>O<sub>2</sub> in the periplasm of eukaryotes and in the endoplasmic reticulum in eukaryotes is emerging as another activity of protein-bound coenzyme Q. Bardwell and coworkers<sup>35,36</sup> identified two thiol proteins, DsbA and DsbB, involved in oxidative formation of protein disulphide bond (Dsb) necessary for export of proteins in *E. coli*. DsbB is a tetraspanin transmembrane protein with Q<sub>8</sub> bound in a loop on the periplasmic side. Purified DsbB protein (20 kDa) is strikingly purple in colour, ascribed to the quinhydrone charge transfer complex<sup>35</sup>. DsbB oxidizes the protein-thiols of DsbA and transfers the electrons through the quinone to reduce O<sub>2</sub> to H<sub>2</sub>O<sub>2</sub>. The quinhydrone (Q : QH<sub>2</sub> = •Q : Q•) of coenzyme Q may act as an intermediate to overcome the spin barrier in electron transfer to O<sub>2</sub>. Mechanism similar to Dsb oxidation seems to operate in NADH-quinone reductase in mitochondrial complex I. Indeed the Q-binding sites in these two systems are alike<sup>36</sup>. Alternative oxidase of plant mitochondria described above fits well with these features. Other reports are appearing on coenzyme Q-bound proteins and these are likely to facilitate its functions and transport.

### Initiation of work on coenzyme Q in India

I returned to IISc in 1959, encouraged by P. S. Sarma, who moved there as professor of biochemistry. A few crystals of coenzyme Q<sub>10</sub> isolated from beef heart mitochondria in the Enzyme Institute that I carried are the first to make an appearance in Bangalore and India. I started work on coenzyme Q. As coenzyme Q was new, whatever we did churned out original information. Our first major finding in 1961 was that coenzyme Q is not exclusively in the mitochondria, but is native to all cell membranes. In the neighbouring laboratory, P. S. Sastry

standardized a procedure for the separation of rat liver cell fractions, and analysis of these by J. Jayaraman showed that a significant portion of cellular coenzyme Q occurred in non-mitochondrial fractions of liver tissue<sup>37</sup> and also, surprisingly, in soluble cytosol, representing protein-bound form. Following this, intracellular distribution was confirmed in another Indian laboratory<sup>38</sup> after our discussion with A. Sreenivasan visiting our laboratory. There were two earlier reports from laboratories of Morton<sup>39</sup> and Rudney<sup>40</sup> indicating that all the coenzyme Q was not recovered in the mitochondria sans recognizing its meaning. With enzyme data to support we affirmed its extra-mitochondrial presence and made a clear statement of a new finding. Again, this finding represents the importance of the person, the place and the time, and was generously described by Crane<sup>2</sup> thus: 'The groundwork for broader functions of coenzyme Q in membranes other than mitochondria was laid at this time when Ramasarma and coworkers (Sastry *et al.*, 1961) showed that coenzyme Q was in other cellular membranes'. Mellors and Tappel<sup>41</sup> soon uncovered its antioxidant function in microsomal membranes. It was further found to be present in the LDL fraction of serum lipoproteins by Stocker *et al.*<sup>42</sup>, where it acted as an antioxidant better than vitamin E. Crane and Morre<sup>43</sup> found that coenzyme Q is enriched in the Golgi apparatus where protein-disulfide formation occurs before the proteins are excreted. Other roles in these sites can be expected to follow. Sinatra<sup>44</sup> listed our finding as the third important event in the development of work on coenzyme Q in the book published in 1999 entitled *The Coenzyme Q10 Phenomenon*. The work at IISc in the first decade, summarized in a review<sup>45</sup> published in the *Journal of Scientific and Industrial Research* (CSIR) in 1968 and the major findings are given below: (1) Coenzyme Q is distributed in all cell components. (2) Exogenous Q given orally is exclusively absorbed into the liver and not in the other tissues, unlike vitamin K and E. (3) All the tissues have independent capacity for synthesis of Q, but not in early developing embryonic tissues. (4) The mevalonate pathway, used for synthesis of Q in animals, plants and fungi, is not operative for synthesis of Q in some bacteria and of vitamin K in mycobacteria. (5) Liver Q accumulates owing to

## HISTORICAL NOTES

lowered catabolism and enhanced synthesis under stress conditions of vitamin A deficiency, cold exposure and thyrotoxicosis. (6) Excess Q in the liver, obtained by synthesis or absorption, inhibits its own synthesis, and also of cholesterol that shares the same biosynthetic pathway, and consequently lowers serum cholesterol.

The focus of our research continued on the actions of exogenous Q at the level of mitochondria, tissues or animals. This led to the finding described below of the relaxation of constricted arterial rings by the side-chain truncated water-soluble metabolite of the lipid-quinone.

### International campaign for clinical applications of coenzyme Q

Coenzyme Q would have remained a biochemistry-delight like coenzymes I (NAD) and II (NADP). But Karl Folkers set out on an 'unrelenting campaign to find nutritional and therapeutic significance' and 'search for a vitamin-like requirement for coenzyme Q', in the words of Crane<sup>2</sup>. A human subject, suffering with megaloblastic marrow, was the first to be administered Q<sub>10</sub> in January 1963 in Jerusalem<sup>46</sup>. Folkers depended on the redox and implication in energy transduction of coenzyme Q in evaluating the effects. He promoted the idea of correction of partial deficiency by supplemented Q, sometimes with small changes not much above biological variation.

Initial clinical studies in the 1970s on therapeutic effects of coenzyme Q supplementation were carried out by Yamamura and others in Japan on cardiovascular disease and hypertension, and Folkers and others elsewhere in the world on a number of nutritional and disease conditions<sup>46</sup>. These studies heralded coenzyme Q as an important molecule with unimagined multiple health benefits. A trend of papers on clinical studies

overshadowing those on basic functions started about the time of the first major international meeting organized by Eisai Co, near Mt. Fuji in Japan in 1976. Limitation for extended clinical studies was overcome in 1964 when Nisshin Co, Japan succeeded in making large quantities of coenzyme Q<sub>9</sub> by chemical synthesis using Q<sub>0</sub> and solanesol (nine isoprene units). Solanesol used in these early studies was obtained from tobacco-leaf lipids imported from Guntur (India), the town where coincidentally I graduated in chemistry. The availability from Eisai Co, Japan of large quantities of the compound made possible clinical studies and its introduction for treatment of congestive heart failure in 1974. Kanegafuchi Co, Japan developed microbiological processes in 1977 for obtaining coenzyme Q<sub>10</sub> in its natural all trans-isomer form. Later other companies in many countries developed processes for large-scale production. Some variable effects of coenzyme Q samples reported in the literature were attributed to cis-isomers in synthetic products. Several clinical investigations followed. Tishcon Corp, USA (Ravi Chopra) developed a range of formulations, including soft gel, and had been gifting samples to support research investigations. Coenzyme Q can now be purchased in kilogram quantities. Many formulations for use in patients are now available over the counter even in some supermarkets around the world. A number of traders offer Q<sub>10</sub> tablets in many cities in India according to the Internet, but they can hardly be found in any neighbourhood pharmacy shops in Bangalore.

In the last three decades several clinical studies reported amazing improvements in patients with a variety of diseases on supplementation with coenzyme Q<sub>10</sub> as tablets or capsules of suitable vehicles orally, usually in the dosages of 30 mg/day or multiples thereof. A comprehensive survey of the formula-

tions that increase plasma Q concentration is available in a review by Bhagawan and Chopra<sup>47</sup>. The enhanced blood concentration above the base line of 1 µg/ml or about 1 µM concentration in the blood was taken as an indicator of bioavailability. A list of these studies (references not cited here) is given in Table 2. Some of the effects are marginal. The clinical community is impressed with the feeling of wellness coenzyme Q-supplements gave to the patients, and prompted its wider use. It is now used more commonly before and after heart operations, and also to relieve statin-related muscular pains. And the list is growing. Folkers' optimism on beneficial biomedical effects of coenzyme Q is vindicated. Will the expanding functions of coenzyme Q make it a vitamin of Folkers' vision?

### How does a lipid-quinone do so many tasks?

Ability to reduce to quinol and to reoxidize to quinone makes coenzyme Q an excellent membrane-located electron carrier. Having a fully substituted ring – one polyprenyl, one methyl and two methoxy groups – allows the molecule little reactivity other than facilitating electron exchange. In contrast to the related isoprenoid-lipids such as vitamin K (methyl-naphthoquinone), vitamin E (trimethyl-chromane) and plastoquinone (dimethyl-benzoquinone), the selection of the two methoxy groups in coenzyme Q, indeed remains an enigma. Emerging from studies on bacterial photosynthetic reactions is an inkling that 2-methoxy group facilitates electron transfer and also binding to proteins<sup>33</sup>. Redox activity alone could not explain the multiple clinical effects. The side chain and methoxy groups of this molecule must be holding clues for other properties awaiting discovery.

**Table 2.** List of conditions where coenzyme Q is reported to give beneficial effects

Cardiovascular diseases	Congestive heart failure	Myocardial infarction
Angina pectoris	Encephalomyopathy	Atherosclerosis
Vascular endothelial function	Hypertension	Amyotrophic lateral sclerosis
Periodontal disease	Gingivitis, gum disease	Muscular dystrophy
Immune deficiency	Asthma	Migraine headache
Freiderich ataxia	Chronic fatigue syndrome	Male infertility
Alzheimer	Huntington	Parkinson
Cancer (breast, lung)	Diabetes (type 2)	Smooth muscle relaxation
Macular degeneration	Obstructive pulmonary disease	Statin treatment

### Absorption and distribution of coenzyme Q

The long lipid side chain influences absorption and catabolism of coenzyme Q in addition to the obvious role in membrane localization. With phytyl side chain, vitamin K<sub>1</sub> (one double bond) and vitamin E (used in cyclizing to chromane ring) of plant origin are absorbed and distributed in several tissues in the rat. With similar redox functions and poly-prenyl side chain (multiple double bonds), bacterial vitamin K<sub>2</sub> is absorbed well, but plant plastoquinone is totally rejected in the rat. Coenzyme Q is absorbed relatively poorly and appears only in the liver and in blood, and even that little is lost on saturating the double bonds in the side-chain. Ubichromenol, a cyclized chromene derivative of ubiquinone was absorbed and distributed in all tissues, similar to vitamin E. Some specificity resides in the side-chain unsaturation and the quinone ring substitutions in absorption and utilization of these compounds in animals<sup>45,48</sup>.

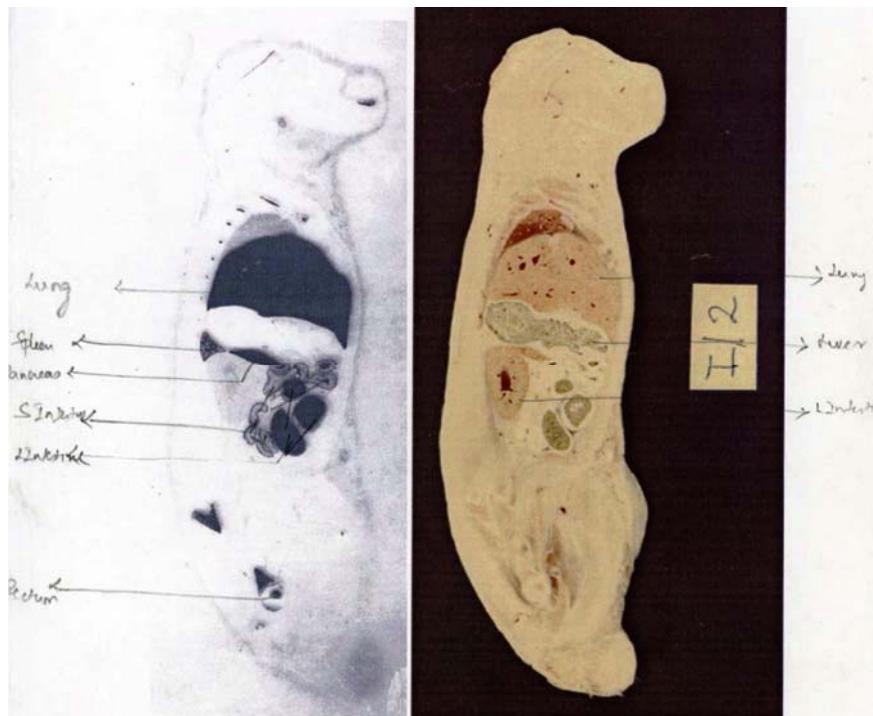
It was of interest to know about absorption, distribution and degradation of the supplemented exogenous lipid-quinone. Coenzyme Q was poorly absorbed through the oral route. Absorbed Q in the rat was found mostly in the liver by Lawson *et al.*<sup>49</sup> in Liverpool and by Rudney and Sugimura<sup>50</sup> in Cleveland, in the early studies in 1961. In 1963, Jayaraman *et al.*<sup>51</sup> confirmed these observations in Bangalore with <sup>14</sup>C-coenzyme Q (labelled in the first two carbon atoms of the side chain attached to the ring, a gift from Hoffman La Roche). Evidence was obtained that only a small fraction (<5%) of the coenzyme Q given orally in oil base was absorbed at 24 h and was found mostly in the liver tissue in the lipid form. Only a small portion appeared in the blood and spleen, but none in the other tissues. Over a period of the next four days, Q decreased in the liver but could not be detected in the other tissues even with sensitive radioactivity measurement, indicating lack of internal redistribution of the exogenous lipid-quinone. Indeed this raised the inconvenient question as to how the effect of coenzyme Q in a tissue can be explained if it does not reach the site.

Injected into the blood of rat, <sup>14</sup>C-Q rapidly moved to the liver within minutes, as most lipids do, and accumulated there with hardly detectable amounts in

other tissues<sup>51</sup>. Similar findings were obtained in Eisai laboratories in Japan with rat and the guinea pig. Given by injection into the veins of the rats (7  $\mu$ Ci/rat), an attempt was made in 1980 by Nakamura *et al.*<sup>52</sup> to show some <sup>14</sup>C-labelled exogenous Q reached the heart after 24 h to justify the observed cardiac effects. But incorporation of radioactivity was in trace amounts in the tissue, with most of the 8000 DPM being present in soluble cytosol. And it is not clear if the blood retained in the tissue was excluded. In 1983, Yuzuriha *et al.*<sup>53</sup>, also showed that in the guinea pig intravenously given <sup>14</sup>C-coenzyme Q<sub>10</sub> (10  $\mu$ Ci/guinea pig), it rapidly decreased from the blood to 5% of the initial level, but rose to 10% by 8 h. Radioactivity in the tissues increased during 24 h, with nearly 90% in the liver and 8% in the urine. This was interpreted as transfer of Q<sub>10</sub> from liver to blood and other tissues at late intervals and is now acceptable in view of the presence of coenzyme Q in blood lipoproteins<sup>54</sup> that are initially packaged in the liver. Note that incorporation of radioactivity from Q<sub>10</sub> was measured in the solubilized tissue sam-

ples in these studies and not in the isolated lipid-quinone. Large presence of radioactivity was found after 20 h in the lungs and some other soft muscle tissues on autoradiography of the cut sections in a mouse given <sup>14</sup>C-coenzyme Q (Figure 4). I am recording this unpublished finding of Hoffman La Roche which Oswald Wiss shared with me during my visit to Basel in 1965 and authorized me to use the figure. I surmise that the radioactivity represents water-soluble derivative products commending research on their identity and functions.

Multiple double bonds present in the side chains of coenzyme Q and vitamin K<sub>2</sub> are apparently necessary for their absorption in animals. These lipid-quinones with fully saturated side chains, obtained by hydrogenation, were not absorbed<sup>45</sup>. In 1964, Joshi *et al.*<sup>55</sup> found that catabolism of the hydrogenated form of coenzyme Q placed in the blood and transferred to the liver also decreased, as shown by its retention in the liver for extended periods. The unusual accumulation of ubiquinone in vitamin A deficiency, a feature that helped in discovery in Liverpool, was found to involve



**Figure 4.** Distribution of <sup>14</sup>C-ubiquinone 20 h after oral administration of 15  $\mu$ Ci in a mouse. Whole body sections were obtained and exposed to X-ray film for a long period to detect radioactivity. (Right) Photograph of the section showing lung, liver and intestines. (Left) Dark areas indicating radioactivity include lungs, spleen, pancreas and large intestines (three major rounded areas below the indicator line.) (Courtesy: Hoffman La Roche, Basel, Switzerland).

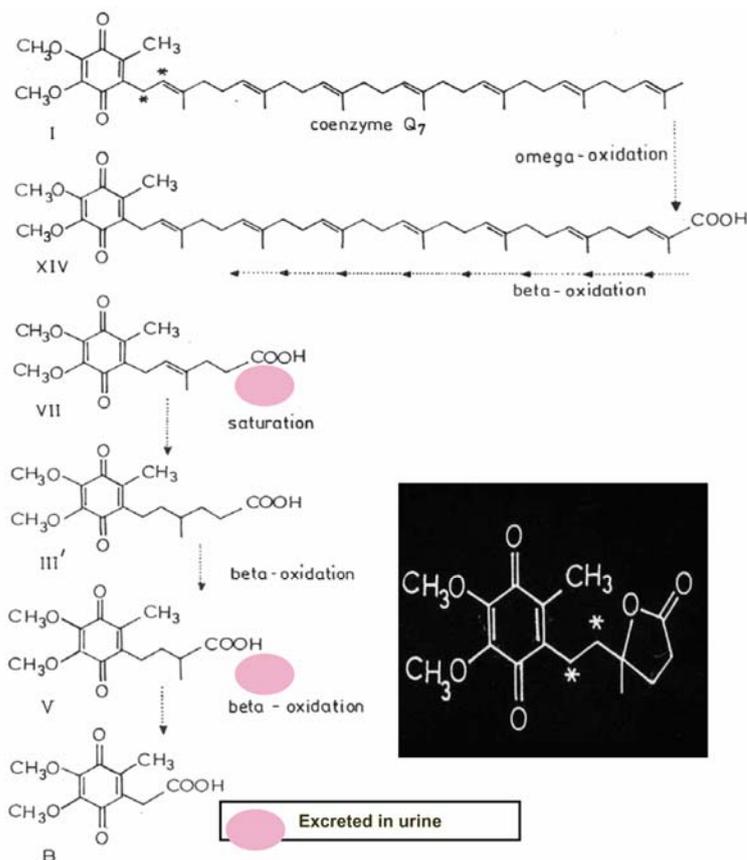
## HISTORICAL NOTES

decreased catabolism in our laboratory. In hindsight, oxidation of unsaturated compounds as a possible function of vitamin A is implicit.

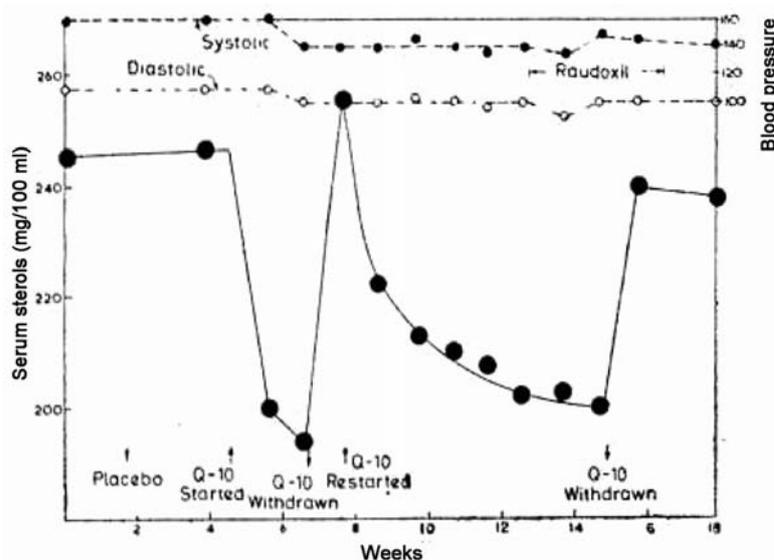
The Hoffman-La Roche team also first showed in 1966 the degradation of Q to a water-soluble quinone with truncated side chain that was excreted in the urine in Q-fed animals (inset, Figure 5). This accounts for derivative products (side chains with 5- and 7-carbon acids, but not shorter) excreted as common detoxification sulphate derivatives in urine described by Gloor *et al.*<sup>56</sup> from Switzerland in 1966 with Q<sub>9</sub>, and Imada *et al.*<sup>57</sup> from Japan in 1970 with Q<sub>7</sub> (Figure 5). It became obvious that part of the catabolism is the truncation of the lipid side chain and excretion of the same water-soluble quinone-acid, formed regardless of the side chain length of the fed Q. Could this lead to its water-soluble polar products of Wiss and of Imada?

Our observation on the relationship of coenzyme Q and hypertension was recorded in 1968 in the *Journal of Scientific and Industrial Research* (India)<sup>45</sup>. The blood pressure of a patient at the Health Centre in IISc, stayed decreased for several weeks, along with a decrease in cholesterol, while on supplement of coenzyme Q<sub>10</sub> (oral dose of 30 mg/day of a syrup-suspension supplied by Hoffman La Roche, given under the supervision of Dr T. B. Subba Rao; Figure 6). This dose of 30 mg was selected, being a rough estimate of total content in an average man. This is perhaps the earliest report on such action of Q<sub>10</sub>. We also tested with a few volunteers from our laboratory, including myself, and found that small decreases were found in the normal levels of blood cholesterol on supplementing Q<sup>58</sup>. We did not pursue this line of work, being unable to take up extended clinical study.

The biochemical rationale of this remarkable effect is explained by our later work. Cholesterol (major) and coenzyme Q (minor) are products of a branched pathway of biosynthesis of isoprene compounds and share the key enzyme, the microsome-based HMGCoA reductase that produces the common precursor, mevalonate. It was already established in many laboratories that this enzyme activity decreased markedly on enriching microsomal membranes with cholesterol when it was fed in the diet. Similarly, coenzyme Q accumulated in the liver on oral supplementation, coinciding with



**Figure 5.** A scheme of degradation of orally administered coenzyme Q. Animals dosed with <sup>14</sup>C-ubiquinone (50; labelled in the first two carbons in the side chain, marked \*) excreted the quinone with lactonized 7-carbon acid in urine (inset)<sup>56</sup>. The quinone acids, 7- and 5-carbon side chains, are excreted in urine<sup>57</sup> from orally dosed coenzyme Q<sub>7</sub>. Crimson spots denote compounds excreted in urine (note the expected final 2-carbon acid had not been detected).



**Figure 6.** Responses of serum cholesterol and blood pressure on oral supplement of coenzyme Q<sub>10</sub> in a hypercholesterolemic man. The person took daily 30 mg Q<sub>10</sub> as a suspension in syrup (courtesy, Hoffmann La Roche, Switzerland) stirred into the cup of coffee in the morning for several weeks as shown. During a break of a week, he attended a marriage and had rich food. The blood pressure and serum sterol concentration were monitored weekly. Both decreased while on Q-treatment<sup>45</sup>.

decreased cholesterol synthesis, albeit only 50%, and thereby its concentration in the blood. This concept of 'feedback' inhibition of the common rate-limiting enzyme of branched pathway by feeding a minor product and thereby decreasing the major product<sup>58</sup> was first introduced in the second International Symposium on Drugs Affecting Lipid Metabolism in Milan, Italy in 1965. Farnesol and other products of the pathway were later found to have similar effect (see Figure 7 for control steps in the isoprene pathway) and such lipid isoprene products that reach and alter the transmembrane domain of the protein are now known to decrease the activity of HMG-CoA reductase. Incidentally it is this enzyme that is competitively inhibited by the statin drugs widely used for controlling hypercholesterolemia. Some of many other useful end-products of this critical step in the pathway, particularly prenylated proteins of which nearly 50 were listed by Roskoski<sup>59</sup>, participate in diverse processes in signal transduction, cell growth, differentiation, cytoskeletal functions and vesicle traffic. And their inhibition by statins may be of concern on long-term treatment of these widely used drugs.

Reports started appearing early the 1970s on large clinical trials on coenzyme Q supplements. Supplements of coenzyme Q (Q<sub>10</sub> and Q<sub>9</sub>, also at a dose per os of 30 mg/day or multiples, coincidentally similar to ours) were first reported by Yamagami *et al.*<sup>60</sup> in 1974, to decrease blood pressure in patients with essential hypertension. Yamamura<sup>61</sup> pioneered these studies and summarized the findings in 1985 from many laboratories, including those in Japanese language. Recent studies of Littaru and coworkers in 1992 in Italy<sup>62</sup>, Langsjoen *et al.*<sup>63</sup> in 1994 in USA and Singh *et al.*<sup>64</sup> in 1999 in India had confirmed the beneficial effect of coenzyme Q in hypertension. These studies on effective decrease in blood pressure with different homologues of coenzyme Q underscore that the length of the side chain is irrelevant, as if it was removed.

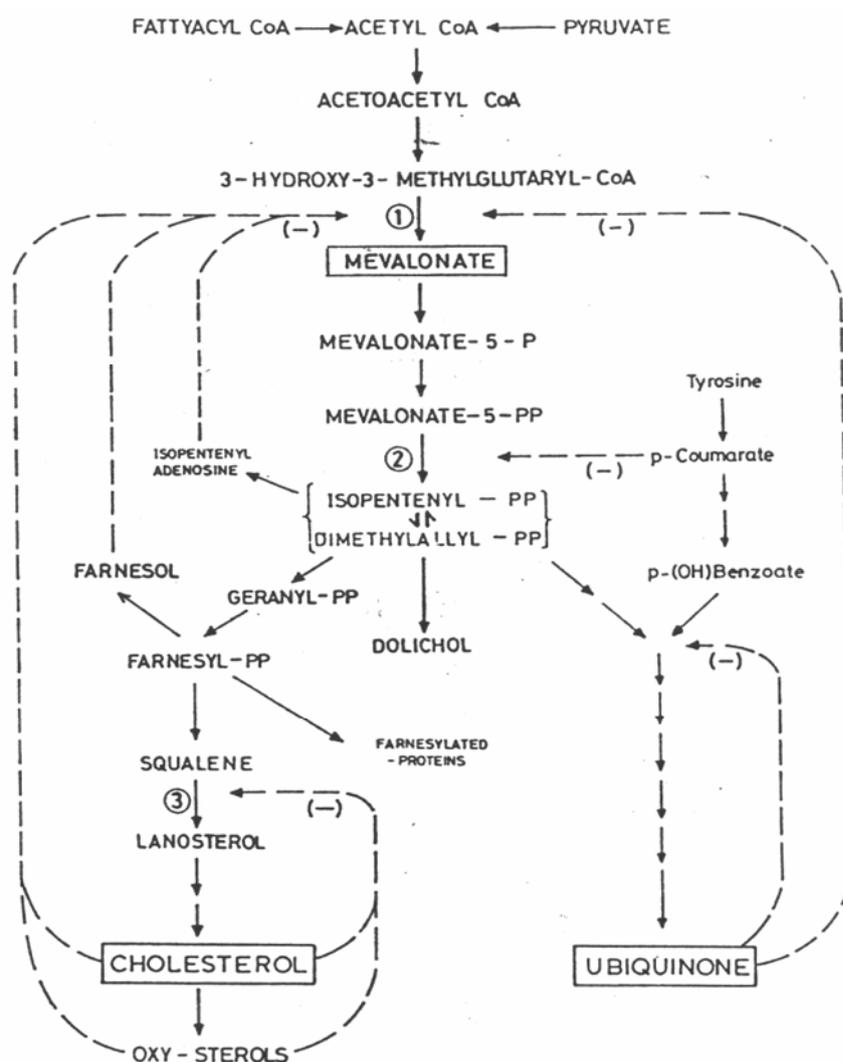
### The link between coenzyme Q and blood pressure – smooth muscle relaxation

Relaxing contracted arteries is a critical step in decreasing elevated blood pres-

sure. Remember, Alfred Nobel was administered by his physician trinitroglycerine, his own famous discovery, to relieve the pain associated with heart condition. Trinitroglycerine and nitrates release nitric oxide, identified as the endothelium-derived relaxing factor (EDRF) by Furchgot and Zawadski<sup>65</sup> in 1980. Nitric oxide (NO) produced in the endothelium is crucial in relaxing the constricted arterial smooth muscle allow-

ing blood flow to the heart tissue. The relaxation effect of NO obtained on activation of nitric oxide synthesizing enzyme (NOS) by agents such as acetylcholine on contracted arterial smooth muscle, explains the reduction of blood pressure.

We found a new activity of a water-soluble form of coenzyme Q obtained on oxidation that explains its effect on blood pressure. Contraction of smooth muscle



**Figure 7.** Regulation of isoprene pathway by possible feedback mechanism. The biosynthetic pathway utilizes acetylCoA (2-carbon) to form the biological isoprene unit, isopentenyl-PP (5-carbon branched methyl compound). This polymerizes head-to-tail to form farnesol-PP (15-carbon), two molecules of which condense tail-to-tail yielding squalene, the 30-carbon hydrocarbon product. Oxidations and rearrangements of squalene lead to the formation of cholesterol, later other oxy-sterols. The side chain of coenzyme Q<sub>10</sub> is formed by head-to-tail condensation of 10 isoprene units. The branching of the pathway is shown to occur at farnesyl-PP in the literature with hardly any evidence. Such large polymeric isoprene side chains are more likely to be built from dimethylallyl-PP and isopentenyl-PP (like the acyl chains in fatty acids) and transferred to the nucleus. The key enzymes in this branched pathway are: (1) hydroxymethylglutaryl-CoA reductase; (2) mevalonate-5-PP decarboxylase and (3) squalene oxidoreductase. The activities of the existing enzymes are decreased (shown by broken arrows) by the end-products in the pathway and other related compounds (pyrophosphate, PP).

## HISTORICAL NOTES

of rat arterial rings by an adrenergic agonist (phenylephrine) and relaxation of this state by acetylcholine or by NO-generating sodium nitroprusside was used as the test system. On ozonolysis the side chain of Q<sub>10</sub> got truncated and water-soluble derivatives were obtained. Bindu *et al.*<sup>66</sup> found in 2003 that these water-soluble acid-products, but not intact lipid-quinone, promoted relaxation of arterial rings (Figure 8). The active products were also derived on treatment of the lipid quinone with ferrous sulphate in the presence of ascorbate or hydrogen peroxide<sup>67</sup> that generates hydroxyl radicals. This relaxation effect was obtained with or without endothelial layer of the rings, indicating that the effect is not through NO as with acetylcholine. The same short-chain product will be obtained with the homologues, Q<sub>10</sub>, Q<sub>9</sub> or Q<sub>7</sub>, tested in these studies. The known independence of the length of the side chain for hypotensive action is thus explained.

The long isoprene side chain of the native coenzyme Q fits the lipid-quinone in the membrane to fulfil its electron transport and antioxidant functions. Innumerable reports are surfacing on the biological effects of supplements of exogenous coenzyme Q. Known only for redox, the lipid form of the quinone is not sufficient to explain these diverse functions. Other derived modified forms have to be invoked. This is schematically indicated in Scheme 2.

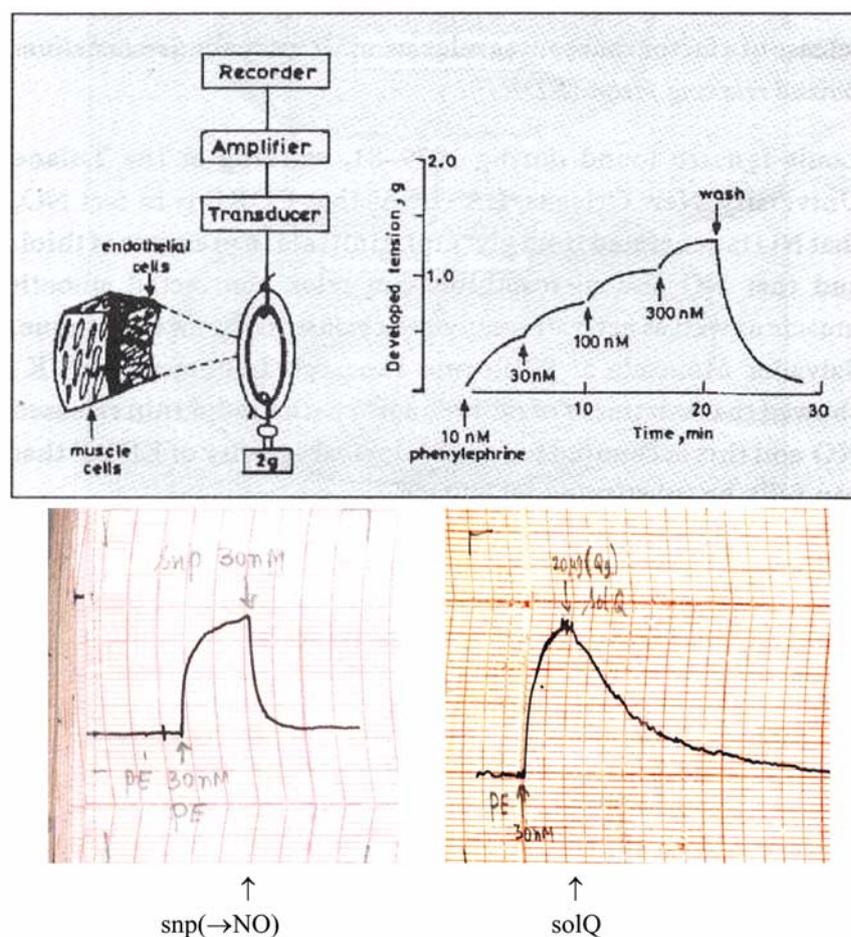
Blood carries coenzyme Q-containing lipoproteins whose oxidation can be expected to coincidentally generate Q-derived relaxing factor (QDRF?) *in situ*. Presence of Q-derived products in the lungs and other tissues, shown in Figure 4, also suggests their possible action in general relaxation processes. Some of the actions described in Table 2 may also involve relaxation of muscular tissues. Coenzyme Q<sub>10</sub> is supplemented to replenish its depletion in the tissues (only liver?) in patients on statin treatment, and improvement of muscle weakness and pain had been hinted. We have a few random observations on relief from the pain of stiff muscles experienced by senior citizens (not on statins) taking coenzyme Q<sub>10</sub> (100 mg/day, tablets: courtesy of Svengenetech, Hyderabad). Arising from the relaxation effect on arterial smooth muscles described above<sup>67</sup>, a general use of coenzyme Q for muscular relaxation is anticipated. And this needs a systematic study. Are we getting closer to calling

ubiquinone vitamin Q? A chronological list of the findings on coenzyme Q/ubiquinone is given in Table 3.

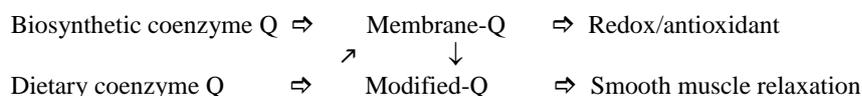
### Coenzyme Q, a vitamin?

Fat-soluble molecules are processed to more polar products to gain biological activity. Do water-soluble forms of coenzyme Q gain wider activities and explain multiple effects now being observed? The exogenously supplied coen-

zyme Q molecule might undergo structural modifications and these derivatives might show functions yet to be discovered. Notice all the fat-soluble vitamins are isoprene structures. Coenzyme Q is related to these in being an isoprenoid (A, K, E, D), having a quinone structure (K) and being able to cyclize to chromenol (E) (Figure 9). A comprehensive definition of a vitamin, with exceptions, will include the following: a small molecular weight organic compound; not synthesized in the body;



**Figure 8.** Relaxation of phenylephrene-contracted arterial rings by water-soluble truncated coenzyme Q<sub>9</sub> derivative. Rings cut from rat arteries were suspended in an organ bath with a weight on one end and the other connected to the transducer that records changes in tension (Grass polygraph) (top left). Step-wise contraction of the rings occurred on addition of increasing concentrations of phenylephrine and this reverted on washing (top right). Relaxation of the contracted ring was obtained on adding NO-releasing sodium nitroprusside (bottom left) and the water-soluble acidic product of coenzyme Q (bottom right).



**Scheme 2.**





**Figure 10.** F. L. Crane, the discoverer of coenzyme Q (right), with the author (left). Photograph taken at the inaugural session of Coenzyme Q 50th Anniversary Conference, Kobe, Japan, 2007.

ubiquinone are chosen remains unthinkable as of now. The quinone also has other tasks. It builds proton gradients and acidifies vacuoles. It forms a charge-transfer complex that aids electron transfer to oxygen (otherwise spin-forbidden) to produce superoxide and hydrogen peroxide, now identified with roles in metabolic regulation, signal transduction, and cellular senescence and apoptosis. It is tempting to invoke a vitamin role for coenzyme Q because of its many features common with fat-soluble vitamins. Only now its deficiencies, albeit only tissue-specific, are being identified, but no syndrome is yet attributed to this lipid-quinone. Supplementing coenzyme Q is now known to provide health benefits, much like nutraceuticals. Early success of clinical studies on cardiovascular conditions dramatically shifted attention of coenzyme Q research to biomedical fields starting at the Eisai Co symposium held near Mt. Fuzi in 1985. Redox alone cannot sustain these multiple effects of coenzyme Q. We are far from understanding how the molecule achieves what it does. Precedence already exists that many active lipid forms are polar derivatives: retinal and retinoic acid from  $\beta$ -carotene, cholecalciferol from cholesterol and inositol-triphosphate from phosphoinositide, a binding protein for modified ring from vitamin K. We proposed that polar product by truncating the side chain are possible active metabolites. This is based on our experience with one such compound active in relaxing of contracted smooth muscle

that explains decreasing blood pressure. In foresight, this approach unfolds a second phase of coenzyme Q research.

Finally I want to refer to the New Year greeting I received from Fred Crane in 2003: 'Q must be good for something, they did a quintuple bypass surgery on me and I was out of the hospital in 5 days, so the CoQ might do some good, stay healthy: Fred'. The discoverer himself derived the benefit of coenzyme Q that he had discovered. I was happy to be with Fred Crane at the Coenzyme Q 50th Anniversary Conference at Kobe, Japan in 2007, to share the first scientific session with him and propose the toast at the reception. A photograph taken at that time (Figure 10) shows Fred in his eighties, compared to the youthful face in 1957 (Figure 1).

1. Crane, F. L., Hatefi, Y., Lester, R. L. and Widmer, C., *Biochim. Biophys. Acta*, 1957, **25**, 220–221.
2. Crane, F. L., *Mitochondrion*, 2007, **78**, S2–S7.
3. Morton, R. A., Wilson, G. M., Lowe, J. S. and Leat, W. M. F., *Chem. Ind.*, 1957, 1649.
4. Morton, R. A., *Nature*, 1958, **182**, 1764–1767.
5. Moore, T. and Rajagopal, K. R., *Biochem. J.*, 1940, **34**, 335–342.
6. Wolf, D. E. *et al.*, *J. Am. Chem. Soc.*, 1958, **80**, 4752.
7. Morton, R. A. *et al.*, *Helv. Chim. Acta*, 1958, **4**, 2343–2357.
8. Gloor, U. and Wiss, O., *Experientia*, 1958, **24**, 410–411.
9. Goldstein, J. L., *Nature Med.*, 2004, **10**, 1015–1018.

10. Green, D. E., In *Ciba Foundation Symposium on Quinones in Electron Transport* (eds Wolstenhome, G. E. W., O'Conner, C. M.), J.A. Churchill, London, 1961, pp. 130–159.
11. Hatefi, Y., Haavik, A. G., Fowler, L. R. and Griffiths, D. E., *J. Biol. Chem.*, 1962, **237**, 2661–2672.
12. Trumpower, B. L., *J. Bioenergy. Biomembr.*, 1981, **13**, 1–24.
13. Mitchell, P., *Fed. Eur. Biochem. Soc. Lett.*, 1975, **59**, 137–139.
14. Swaroop, A. and Ramasarma, T., *Biochem. J.*, 1981, **194**, 657–665.
15. Boveris, A., Cadenas, E. and Stoppani, A. O., *Biochem. J.*, 1976, **156**, 435–444.
16. Storey, B. T., *Plant Physiol.*, 1976, **58**, 521–525.
17. Rich, P. R., Boveris, A., Bonner Jr, W. D. and Moore, A. L., *Biochem. Biophys. Res. Commun.*, 1976, **1**, 695–703.
18. Bhate, R. H. and Ramasarma, T., *Arch. Biochem. Biophys.*, 2009, **486**, 165–169.
19. Møller, I. M., Rasmussen, A. G., Siedow, J. N. and Vanlerberghe, G. C., *Arch. Biochem. Biophys.*, 2010, **495**, 93–94.
20. Bhate, Radha H. and Ramasarma, T., *Arch. Biochem. Biophys.*, 2010, **495**, 95–96.
21. Bhate, Radha, H. and Ramasarma, T., *Indian J. Biochem. Biophys.*, 2010, **47**, 306–310.
22. Raj, R. K., Puranam, R. S., Kurup, C. K. R. and Ramasarma, T., *Biochem. J.*, 1988, **256**, 559–564.
23. Mohan, S., Pradeep, C. G., Abilash Kumar, R., Jayakumar, K. and Kalesya Raj, R., *Biochem. Biophys. Res. Commun.*, 2001, **288**, 949–953.
24. Jonassen, T., Beth, N., Marbois, K., Faull, F., Clarke, Catherine, F. and Larsen, P. L., *J. Biol. Chem.*, 2002, **277**, 45020–45027.
25. Larsen, P. L. and Clarke, Catharine, F., *Science*, 2002, **295**, 120–123.
26. Chatterjee, Nirupama, Kiran, S., Ram, B. M., Islam, Nashreen, S., Ramasarma, T. and Ramakrishna, Gayatri. *Mech. Ageing Death*, 2011, **132**, 230–239.
27. Giorgio, M., Trinei, M., Migliaccio, E. and Pellicci, P. G., *Nat. Rev. Mol. Cell Biol.*, 2007, **8**, 722–728.
28. Ramasarma, T. and Lester, R. L., *J. Biol. Chem.*, 1959, **234**, 672–676.
29. Gupte, Sharmila, Wu, E.-S., Hoechli, Luzia, Hoechli, M., Jacobson, K., Sowers, A. E. and Hackenbrock, C. R., *Proc. Natl. Acad. Sci. USA*, 1984, **81**, 2606–2010.
30. King, T. E., In *Coenzyme Q: Biochemistry, Bioenergetics and Clinical Applications of Ubiquinone* (ed. Lenaz, G.), John Wiley, 1985, pp. 391–408.
31. Yu, C. A., Yu, Linda and King, T. E., *Biochem. Biophys. Res. Commun.*, 1977, **78**, 259–265.

32. Suzuki, H. and Ozawa, T., *Biochem. Biophys. Res. Commun.*, 1986, **138**, 1237–1242.
33. Wright, C. A., Nakkasoglu, A. S., Poluektov, Y., Mattis, A. J., Nihan, D. R. and Lipshutz, B. H., *Biochim. Biophys. Acta*, 2008, **1777**, 7–8.
34. Echtay, K. S., Winkler, E. and Klingenberg, M., *Nature*, 2000, **408**, 609–613.
35. Regeimbal, J., Gleiter, S., Trumpower, B. L., Yu, C. A., Diwakar, M., Ballou, D. P. and Bardwell, J. C. A., *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 13779–13784.
36. Xie, T., Yu, Linda, Bader, M. W., Bardwell, J. C. A. and Yu, C. A., *J. Biol. Chem.*, 2002, **277**, 1649–1652.
37. Sastry, P. S., Jayaraman, J. and Ramasarma, T., *Nature*, 1961, **189**, 577–580.
38. Aiyar, A. S. and Sreenivasan, A., *Nature*, 1961, **190**, 344.
39. Hemming, F. W., Pennock, J. F. and Morton, R. A., *Biochem. J.*, 1958, **68**, 29P.
40. Slater, E. C., Rudney, H., Bouman, J. and Links, J., *Biochim. Biophys. Acta*, 1961, **47**, 497–514.
41. Mellors, A. and Tappel, A. L., *Lipids*, 1966, **1**, 282–284.
42. Stocker, R., Bowry, V. W. and Frei, B., *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 1646–1650.
43. Crane, F. L. and Morre, D. J., In *Biomedical and Clinical Aspects of Coenzyme Q* (eds Folkers, K. and Yamamura, Y.), 1977, pp. 3–14.
44. Sinatra, S. T., *The Coenzyme Q<sub>10</sub> Phenomenon*, McGraw Hill Co, USA, 1999.
45. Ramasarma, T., *J. Sci. Ind. Res.*, 1968, **27**, 147–164.
46. Folkers, K., In *Coenzyme Q* (ed. Lenaz, G.), John Wiley, 1985, pp. 457–478.
47. Bhagawan, H. N. and Chopra, R. K., *Mitochondrion*, 2007, **78**, 878–888.
48. Ramasarma, T., *Adv. Lipid Res.*, 1968, **6**, 107–180.
49. Lawson, D. E. M., Threlfall, D. R., Glover, J. and Morton, R. A., *Biochem. J.*, 1961, **79**, 201.
50. Rudney, H. and Sugimura, T., In *Ciba Foundation Symposium on Quinones in Electron Transport* (eds Wolstenholme, G. E. W. and O'Connor, C. M.), J. A. Churchill Ltd, London, 1961, pp. 211–213.
51. Jayaraman, J., Joshi, V. C. and Ramasarma, T., *Biochem. J.*, 1963, **88**, 369–373.
52. Nakamura, T., Sanma, H., Himeno, M., and Kato, K., In *Biomedical and Clinical Aspects of Coenzyme Q* (eds Yamamura, Y., Folkers, K. and Ito, Y.), Elsevier/North-Holland Biomedical Press, Amsterdam, 1980, vol. 2, pp. 3–14.
53. Yuzuriha, T., Takeda, M. and Katayama, K., *Biochim. Biophys. Acta*, 1983, **759**, 280–291.
54. Alleva, R., Tomasetti, M., Bettino, M., Curatolo, G., Littarru, G. P. and Folkers, K., *Proc. Natl. Acad. Sci. USA*, 1995, **92**, 9388–9391.
55. Joshi, V. C., Jayaraman, J., Kurup, C. K. R. and Ramasarma, T., *Indian J. Biochem.*, 1964, **1**, 7–12.
56. Gloor, U., Wursch, H., Mayer, O., Isler, O. and Wiss, O., *Chim. Helv. Acta*, 1966, **49**, 2582–2589.
57. Imada, I., Watanabe, M., Matsumoto, N. and Morimoto, H., *Biochemistry*, 1970, **9**, 2870–2878.
58. Ramasarma, T., Joshi, V. C., Inamdar, A. R., Aithal, H. N. and Krishnaiah, K. V., *Prog. Biochem. Pharmacol.*, 1966, **2**, 56–61.
59. Roskoski Jr, R., *Biochem. Biophys. Res. Commun.*, 2003, **303**, 1–7.
60. Yamagami, T., Iwamoto, Folkers, K. and Bloomquist, C. G., *Int. J. Vitamin Res.*, 1974, **44**, 487–496.
61. Yamamura, Y., In *Coenzyme Q* (ed. Lenaz, G.), John Wiley, 1985, pp. 479–485.
62. Digiesi, V., Cantini, F., Bisi, G. C., Guarino, A., Oradei and Littarru, G. P., *Curr. Ther. Res.*, 1992, **51**, 668–672.
63. Langsjoen, P., Lanjoen, R., Willis, R. and Folkers, K., *Mol. Aspects Med.*, 1994, **15**, S265–S272.
64. Singh, R. B., Niaz, M. A., Rastogi, S. S., Shukla, P. K. and Thakur, A. S., *J. Hum. Hypertens.* 1999, **13**, 203–208.
65. Furchgot, R. F. and Zawadski, J. V., *Nature*, 1980, **283**, 373–376.
66. Bindu, R., Venkataraman, B. V., Aparna V. S. Rao and Ramasarma, T., *BioFactors*, 2003, **18**, 137–143.
67. Bindu, R., Venkataraman, B. V., Aparna V. S. Rao and Ramasarma, T., *Indian J. Biochem. Biophys.*, 2003, **40**, 5–13.
68. Quinzio, C. M., Hirano, M. and Dimauro, S., *Mitochondrion*, 2007, **7S**, S122–S12.
69. Sekhar, B. S., Kurup, C. K. R. and Ramasarma, T., *Mol. Cell. Biochem.*, 1990, **92**, 147–157.
70. Crane, F. L., Sun, I. L., Crowe, R. A., Alcaín, F. J. and Low, H., *Mol. Aspects Med. Suppl.*, 1994, **15**, s1–s11.
71. Kishi, T., Morre, D. M. and Morre, D. J., *Biochim. Biophys. Acta*, 1999, **1412**, 66–77.
72. Gille, L. and Nohl, H., *Arch. Biochem. Biophys.*, 2000, **375**, 347–354.

ACKNOWLEDGEMENTS. I thank the Indian National Science Academy, New Delhi for the position of Honorary Scientist. Much of the work in my laboratory was carried out in the Department of Biochemistry, Indian Institute of Science, Bangalore, and I thank many of the colleagues and students there, and collaborators elsewhere. In this historical perspective, I recorded items that would not have found a place elsewhere such as 'ubiquinone', solanesol from Guntur, India, and Crane-administered coenzyme Q, his own discovery. I selected a limited number of references wherein the original ideas were presented, and not extensions of earlier work (without acknowledgement in some cases). Some of these are unjustifiably ignored in the mainstream literature and I believe this gives authors the deserved credit and historical advantage. I dedicate this article to the memory of David Green, who gave me the opportunity to work with the just-discovered coenzyme Q in 1957 and enter the field of biological oxidations.

*T. Ramasarma is associated with the Department of Biochemistry, Indian Institute of Science, Bangalore 560 012, India and Centre for DNA Fingerprinting and Diagnostics, Hyderabad 500 001, India.*  
e-mail: ramasarma\_1932@rediffmail.com