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Review Article

THE PARTICIPATION OF COENZYME Q IN FREE RADICAL PRODUCTION AND ANTIOXIDATION

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Abstract—Published experimental data pertaining to the participation of coenzyme Q as a site of free radical formation in the mitochondrial electron transfer chain and the conditions required for free radical production have been reviewed critically. The evidence suggests that a component from each of the mitochondrial NADH-coenzyme Q, succinate-coenzyme Q, and coenzyme QH_2-cytochrome *c* reductases (complexes I, II, and III), most likely a nonheme iron-sulfur protein of each complex, is involved in free radical formation. Although the semiquinone form of coenzyme Q may be formed during electron transport, its unpaired electron most likely serves to aid in the dismutation of superoxide radicals instead of participating in free radical formation. Results of studies with electron transfer chain inhibitors make the conclusion dubious that coenzyme Q is a major free radical generator under normal physiological conditions but may be involved in superoxide radical formation during ischemia and subsequent reperfusion. Experiments at various levels of organization including subcellular systems, intact animals, and human subjects in the clinical setting, support the view that coenzyme Q, mainly in its reduced state, may act as an antioxidant protecting a number of cellular membranes from free radical damage.

Keywords—Coenzyme Q, Ubiquinone, Free radicals, Lipid peroxidation, Antioxidant, DT diaphorase, Superoxide dismutase, Review

INTRODUCTION

Coenzyme Q is an exceedingly versatile molecule functionally involved in a number of distinct, but related, cellular processes. Coenzyme Q, the trivial name for 2,3-dimethoxy-5-methyl-6-multiprenyl-benzoquinone (Fig. 1), has also been called Q_{275}, mitoquinone, and ubiquinone. We prefer the trivial name coenzyme Q to ubiquinone for the following reasons: i) The term ubiquinone implies that the compound is ubiquitous in biological systems.[1] It is not present in a number of organisms[2] including gram-positive bacteria,[3] at least one species of photosynthetic bacterium,[4] and some fungi.[5] It is also not present in methanogenic bacteria;[6] ii) a coenzymic function for this benzoquinone, originally suggested by Green,[7] has received considerable experimental support[8–10] as a result of the isolation of mitochondrial coenzyme Q apoproteins or binding proteins; iii) objection to the use of the term coenzyme Q on the basis that it may be confused by students of biochemistry with a coenzyme of Q-enzyme[2] is no longer valid as this term for amylo (1,4–1,6)-transglycolase is no longer used in modern texts of biochemistry. A survey of recent textbooks of biochemistry indicates that the designation coenzyme Q is preferred; and iv) The term ubiquinone is easily confused with ubiquitin, a 9000-dalton, heat-stable

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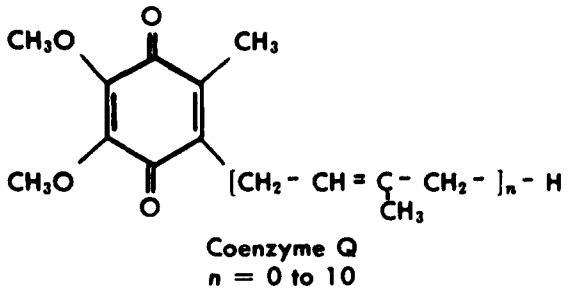

Fig. 1. The structure of coenzyme Q.

peptide[11] involved in the regulation of reticulocyte proteolytic systems as well as the insertion of monoamine oxidase B into the outer mitochondrial membrane.[12] Logical arguments for general acceptance of the coenzyme Q terminology have been presented elsewhere.[13-15]

Coenzyme Q was first discovered as an obligatory component of the mitochondrial succinoxidase system by Crane et al.[16] in 1957 and was later shown by Szarkowska[17] to be involved similarly in the mitochondrial NADH oxidase pathway. These conclusions were substantiated by Norling et al.[18] in experiments involving a quantitative reincorporation of the quinone in coenzyme Q-depleted submitochondrial particles. Direct observation of coenzyme Q oxidation-reduction cycles during electron transport function have been reported.[19] Coenzyme Q thus provides a link allowing reversible interactions between the NADH dehydrogenase, succinate dehydrogenase, and cytochrome *b*-c_1 portions of the electron transport chain[20-22] (Fig. 2). A number of other dehydrogenases donate reducing equivalents to the cytochrome system via coenzyme Q, two examples of which are alpha-glycerophosphate dehydrogenase and ETF dehydrogenase. In the reduced state, coenzyme Q exerts an influence on the rate of energy liberation from the electron transport chain by regulation of the activities of the succinate dehydrogenase,[23-26] NADH dehydrogenase,[27,28] and cytochrome *b*-c_1[29-31] complexes. Coenzyme Q also appears to be involved in the establishment of an inner mitochondrial transmembrane protonmotive gradient, the "protonmotive Q cycle,"[32-34] resulting in the conservation of energy released by oxidoreduction reactions of the electron transport chain (Fig. 3). In addition, coenzyme Q has been implicated in both the production of damaging free radicals and as an antioxidant protecting the components of the membrane in which it resides from peroxidative damage. This review will examine evidence regarding the involvement of coenzyme Q in free radical production during mitochondrial electron transfer chain activity and the sites in which this occurs. In addition, evidence supporting and consistant with an antioxidant role for coenzyme Q will be presented. These subjects have been reviewed previously.[36-39]

LIPID PEROXIDATION AND FREE RADICAL FORMATION BY THE MITOCHONDRIAL ELECTRON TRANSFER CHAIN

Free radicals are formed during activity of the mitochondrial electron transfer chain.[40] The rate of superoxide radical formation is directly proportional to the rate of mitochondrial oxygen utilization.[40] The generation of strong oxidizing agents from $O_2^{\cdot-}$,[41] which in the presence of iron in several forms promote and propagate lipid peroxidation, results in altered membrane function and protein denaturation[42] and thus poses a constant threat to cellular homeostasis. These topics have been the subject of extensive review[43-46] and an opposing view has been published.[47]

Studies of mitochondrial swelling have shown that two types of such volume changes occur: those which are reversible[48] and those which are irreversible. Swelling may be initiated by agents such as thyroxine and phosphate[49] and reversed by ATP and the radical scavenger manganese ion.[48] Irreversible swelling may be initiated by prooxidants such as glutathione,[50] ascor-

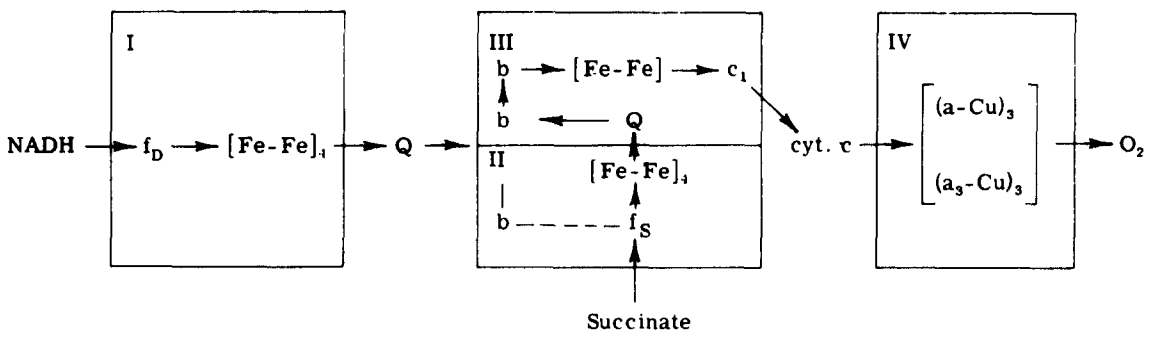

Fig. 2. Coenzyme Q (UQ) in the mitochondrial electron transfer chain.

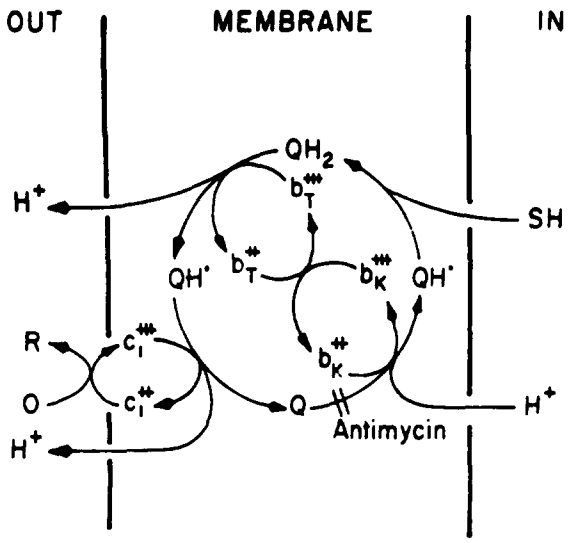

Fig. 3. Electron transfer and protonmotive reactions of coenzyme Q and the cytochrome b–c_1 complex. [From[35] with permission.]

bate, and iron and is inhibited by the antioxidant alpha-tocopherol.[51] Prooxidant-induced swelling has been shown to be associated with the production of thiobarbituric acid reactive compounds,[51] thus implicating the occurrence of lipid peroxidation. Although an involvement of active oxygen in this process was suspected, no assay for the superoxide radical existed before 1969. The discovery by McCord and Fridovich[52] of the enzyme superoxide dismutase catalyzing the removal of superoxide by a dismutase reaction,

$$2O_2^{\cdot-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \qquad (1)$$

allowed a direct connection to be established. Loeschen et al.[53] and Boveris and Cadenas[54] were the first to demonstrate the production of $O_2^{\cdot-}$ by the electron transport chain by comparison of H_2O_2 formation in the presence and absence of SOD. Table 1 shows the rates of H_2O_2 and superoxide production by submitochondrial particles in the presence of succinate and a concentration of antimycin sufficient to completely block the flow of electrons over the chain. Boveris and Chance[40] had previously observed a high rate of H_2O_2 production by pigeon heart mitochondria respiring with malate + glutamate and succinate + malate as substrates. The rate of H_2O_2 production was elevated by increasing oxygen pressure or by the addition of antimycin and uncouplers of oxidative phosphorylation.[40] Participation of the reduced state of oxidation-reduction components of the electron transfer chain in superoxide radical formation was implicated by the demonstration[55] that mitochondrial H_2O_2 production occurs during state 4 respiration and ceases upon transition to state 3. These results, in addition to experiments with electron transfer chain inhibitors, narrowed the site(s) of superoxide production to reduced members of the respiratory chain on the substrate side of the antimycin block, that is, flavoprotein, nonheme iron-sulfur protein, coenzyme Q, and cytochrome b. Trumpower and Simmons[56] confirmed the localization of a superoxide-generating site in the span [succinate dehydrogenase to cytochrome b] by demonstrating that the radical could be produced by purified complexes II and III (succinate-cytochrome c reductase). They postulated[56] a mechanism in which the semiquinone form of coenzyme Q gave rise to the superoxide radical. Such a role for coenzyme Q in the production of free radicals has been discussed by Boveris and Cadenas[57] and Ernster and Nelson.[58] Boveris and Cadenas[57] and Forman and Boveris[44] have suggested that the semiquinone form of coenzyme Q, that is, $CoQ^{\cdot-}$ or $CoQH^{\cdot}$, is the major mitochondrial autoxidizable component yielding the superoxide radical. The presence of the semiquinone form of coenzyme Q had first been demonstrated[59] by measuring the semiquinone electron spin resonance signal of coenzyme Q-depleted and coenzyme Q-replenished submitochondrial particles. These observations have been confirmed[60] and the semiquinone form of coenzyme Q has been observed recently in the NADH-CoQ reductase region of the mitochondrial respiratory chain.[61]

Coenzyme Q has been implicated in the etiology of free radical pathology following ischemic episodes[62] and in superoxide anion production during the respiratory burst of human neutrophils by a nonmitochondrial NADH oxidase system.[63] However, it should be noted that no direct evidence has been presented in the studies cited above that coenzyme Q, in any form, is the chemical species involved in superoxide radical

Table 1. H_2O_2 and $O_2^{\cdot-}$ Production Catalyzed by Submitochondrial Particles (SMP)[54]

Source of SMPs	Cytochrome c Reduction, (nmol/min/mg Protein)	Sensitivity to SOD (%)	$O_2^{\cdot-}$ nmol/min/mg	H_2O_2 Protein
Beef heart	6.3	70	4.4	2.5
Rat heart	8.0	62	4.9	3.2
Rat heart	12.8	54	6.9	3.2

formation. These studies on mitochondria and submitochondrial particles implicate a site of superoxide production located between succinate or NADH and cytochrome *b*, but do not discriminate between the several electron-transferring components involved. The report of coenzyme Q involvement in free radical-induced pathology following ischemic episodes[62] is also vague regarding evidence showing that coenzyme Q is responsible. Human neutrophils, which are deficient in cytochromes *a* and *c*, have been reported to possess a flavin-containing NADPH oxidase, a non-mitochondrial *b* type cytochrome, and coenzyme Q_{10}.[64] Based on the stimulation of both oxygen consumption and superoxide radical production by short isoprenoid chain coenzyme Q analogues and inhibition of both events by quinone antagonists, it has been concluded[64,65] that the endogenous coenzyme Q is necessary for oxygen metabolism and microbicidal events in neutrophils. However, the data,[63-65] although strongly suggesting a role for coenzyme Q in superoxide radical and H_2O_2 production in neutrophils, do not exclude a role for other components such as cytochrome *b*, flavin semiquinone, or nonheme iron-sulfur proteins which may also participate in neutrophil electron transfer reactions and superoxide production. This possibility should receive serious consideration as iron-sulfur flavoproteins,[66] flavoproteins,[67] and nonheme iron-sulfur proteins,[68] in addition to quinols,[69,70] have all been shown to have the capacity to generate superoxide radicals. The role of coenzyme Q in the generation of microbicidal radicals by this system has been questioned by the demonstration that exogenous coenzyme Q_{10} acts as an effective quencher of singlet oxygen generated by human leukocytes.[71] In addition, the demonstration by Lutter et al.[72] of a lack of coenzyme Q in enucleated human neutrophils excludes a role for this compound in the generation of bactericidal oxygen species by neutrophils.

More convincing evidence supporting the notion of superoxide production and ensuing hydrogen peroxide formation from mitochondrial coenzyme Q has been presented by Boveris et al.,[73] who report that extraction of submitochondrial particles with acetone, which removes coenzyme Q and a variety of other lipids as well as cytochrome *c*, decreased the rate of superoxide production with succinate as substrate. Reconstitution of extracted membranes with phospholipid and coenzyme Q resulted in a partial restoration of superoxide and hydrogen peroxide production (Table 2), although the degree of restoration was marginal: 14% and 5% for production of $O_2^{\cdot-}$ and H_2O_2, respectively. These authors also report[73] a linear relationship between reducible coenzyme Q reincorporated into the membranes and the rate of peroxide production in the presence of superoxide dismutase, providing additional evidence for a role of functional coenzyme Q in superoxide production. Boveris et al.[73] suggest that a reduced form of coenzyme Q, either the quinol or semiquinone, functions as a univalent reductant of oxygen, namely:

$$CoQH_2 + O_2 \longrightarrow CoQH^{\cdot} + H^+ + O_2^{\cdot-} \quad (2)$$

$$CoQH^{\cdot} + O_2 \longrightarrow CoQ + H^+ + O_2^{\cdot-} \quad (3)$$

Despite this strong evidence for the involvement of coenzyme Q in radical production, the reestablishment of electron flow capabilities by the reincorporation of coenzyme Q into the electron transfer chain also involves participation of all of the electron transfer chain components between succinate and the antimycin block. These experiments leave open the question of their participation in oxy-radical formation. That Boveris et al. are cognizant of this possibility is evident from their concluding remark:[73] "The present experimental data point to ubisemiquinone and ubiquinol as the main sources of H_2O_2 in mitochondrial inner membrane, but the function of other H_2O_2 generators is not excluded."

Using electron spin resonance spectrographic data, Ksenzenko et al.[74] have demonstrated that the superoxide radical is formed from the CoQ semiquinone under nonphysiological conditions in which the submitochondrial electron transfer chain is completely blocked by inhibitors which interfere with the oxidation of $CoQH_2$ by its physiological oxidant ferricytochrome *b*-566. These researchers have also demonstrated[74] that under conditions in which cytochrome *b*-566 becomes highly reduced in the aerobic steady state, that is, when it can no longer compete with O_2 for $CoQ^{\cdot}$, the CoQ semiquinone transfers its unpaired electron to O_2 to form $O_2^{\cdot-}$. Sugioka et al.[75] have also shown a direct involvement of coenzyme Q in superoxide radical formation by using the one-electron NADPH-CoQ_1-NADPH-dependent cytochrome P-450 reductase system as a model system to study the mechanism of coenzyme Q oxidation and reduction. One electron reduction of CoQ_1 produces $CoQ_1^{\cdot-}$ and $O_2^{\cdot-}$.

Table 2. Superoxide Production by Acetone-extracted and Control Mitochondrial Membranes[73]

		Activity (nmol/min/mg Protein)		
Assay*	SOD	SMPs	CoQ-reconstituted	%
$O_2^{\cdot-}$	–	3.89	0.54	14
	+	0.09	0.06	—
H_2O_2	–	1.86	0.04	—
	+	3.72	0.17	5

*Succinate and antimycin present.

The $CoQ_1^{\cdot-}$ so produced can be completely eliminated by SOD as is the case with $CoQ_{10}^{\cdot-}$ formed in submitochondrial particles.[75] It is of interest in this context that SOD does not eliminate the flavin radical produced in complex II, the succinate dehydrogenase complex.[76]

The kinetic data of Sugioka et al.[75] suggest that an equilibrium reaction of the general type

$$CoQ_1^{\cdot-} + O_2 \underset{k_2}{\overset{k_1}{\rightleftarrows}} CoQ_1 + O_2^- \qquad (4)$$

would occur at physiological pH. The rate constant k_2 would be considerably larger than k_1, supporting the concept that $CoQ^{\cdot-}$ may be formed from O_2^- generated by other one electron-reduced components of the electron transfer chain such as FMN or FAD semiquinones or nonheme iron proteins of complex I, II, or III. Sugioko et al.[75] have also shown that at physiological pH, autooxidation of CoQ_1H_2 according to the reaction:

$$CoQ_1H_2 + O_2 \longrightarrow H_2O_2 + CoQ_1 \qquad (5)$$

is extremely slow, and that it consists of two consecutive reactions:

$$2CoQ_1H_2 + 2O_2 \longleftrightarrow 2\,CoQ_1^{\cdot-} + 4H^+ + 2O_2^- \qquad (6)$$

which is extremely slow and

$$2CoQ_1^{\cdot-} + 2H^+ \longrightarrow CoQ_1H_2 + CoQ_1 \qquad (7)$$

which is thermodynamically favorable. These data would appear to support the notion that the CoQ semiquinone radical, when formed, is rapidly dismutated to the more stable fully oxidized and reduced forms. Application of these concepts to the situation in the intact mitochondrial system is an open question as the coenzyme Q radical formed in these two systems differs with respect to hyperfine splitting structure, most likely due to mitochondrial coenzyme Q being bound to and stabilized by apoproteins.[76] This and other aspects of this question have been discussed in detail by de Vries et al.[77,78]

The existence of distinct coenzyme Q binding proteins, or apoproteins, in complexes I, II, and III exhibiting three types of coenzyme Q semiquinone radical signals[79,80] and sensitive to antimycin, thenoyltrifluoroacetone, and rotenone[80] has been well documented. The actuality of such proteins, originally suggested by Green[7] in 1959 and affirmed by Mitchell[81] in 1976, was detected as coenzyme Q semiquinone radicals in purified coenzyme Q-cytochrome *c* reductase[82,83] from beef heart in 1977 and has been identified in yeast mitochondrial coenzyme Q-cytochrome *c* reductase.[84] The presence of such coenzyme Q apoproteins appears to stabilize the coenzyme Q semiquinone form[76] and thus renders it less reactive with O_2.

Convincing evidence for the site of free radical formation in the electron transfer chain has been published by Takeshige et al.[85] They have shown[85] that submitochondrial particles prepared from beef heart catalyze NADH- and NADPH-dependent lipid peroxidation in the presence of ferric ions and ADP. They have also demonstrated that the site of NAD(P)H-dependent production of $O_2^{\cdot-}$ by the respiratory chain is located within the NADH dehydrogenase (complex I) between a mercurial-sensitive and a rotenone-sensitive site,[86] most likely a nonheme iron-sulfur function. The localization of radical formation resulting in lipid peroxidation, measured as malondialdehyde (MDA) formation, in beef heart submitochondrial particles is shown in Table 3. These data indicate that NADH-dependent lipid peroxidation is increased two- to three-fold by *n*-ethylmaleimide and rotenone and inhibited by antimycin and KCN. A direct effect of KCN and antimycin on lipid peroxidation was obviated by their simultaneous addition with rotenone. Turrens and Boveris[87] have confirmed the effect of rotenone on the rate of superoxide production. These data demonstrate that a respiratory chain component between the substrate site and the rotenone-sensitive site of NADH dehydrogenase is required for the reactions leading to lipid peroxidation and that the redox state of the components between the sites inhibited by rotenone and antimycin, that is, coenzyme Q and cytochrome *b*, strongly influences lipid peroxidation. The absence of inhibition of MDA formation during NADPH oxidation has been explained by the maintenance of the oxidized state of electron transport chain components resulting from the relatively low rate of electron flow from NADPH to NAD^+ via transhydrogenase.[88] Takeshige et al.[89] have shown that during NADPH oxidation catalyzed by submito-

Table 3. Effect of Respiratory Chain Inhibitors on MDA Formation by Submitochondrial Particles[89]

Additions	MDA Formation (nmol/min/mg Protein)	
	NADH	NADPH
None	2.54	5.10
n-ethylmaleimide	5.41	5.15
Rotenone	7.11	5.30
Antimycin	0.25	4.54
KCN	0.38	5.30
Rotenone + antimycin	6.12	4.44
Rotenone + KCN	6.86	5.00

chondrial particles, 5% of the endogenous coenzyme Q is reduced in the presence of antimycin, while during NADH oxidation about 80% of the coenzyme Q is reduced. These observations, together with the demonstration that submitochondrial particles from which coenzyme Q has been extracted retain the capacity to form superoxide radicals at a rate equivalent to that of unextracted particles,[89] render difficult the conclusion that coenzyme Q is the principal electron transport chain component involved in superoxide radical formation, especially under physiological conditions.

ANTIOXIDANT PROPERTIES OF COENZYME Q

Evidence in support of an antioxidant role for coenzyme Q has been obtained from experiments performed at several levels of organization ranging from phospholipid vesicles, reconstituted membrane systems, submitochondrial particles, mitochondria, microsomes, and intact cells, to observations on intact animals, as well as observations made in the clinical setting.

Subcellular systems

Evidence of a direct nature in support of an antioxidant role for coenzyme Q has been derived from a number of studies using subcellular systems. The earliest indications that coenzyme Q may function as an antioxidant were by Lea and Kwietny[90] and Mellors and Tappel[91] who showed that both the quinone and quinol forms of coenzyme Q possessed about 20% of the antioxidant activity of alpha-tocopherol against lipid peroxidation. However, antioxidant activity was assayed under high oxygen concentrations in these studies, conditions favoring the spontaneous oxidation of quinol to quinone. Mellors and Tappel[92] subsequently tested coenzyme Q_6 in both the oxidized and reduced forms for antioxidant activity in the presence of lower, more physiological concentrations of oxygen comparable to those in vivo. The data derived from these experiments showed that reduced coenzyme Q_6 was four times more effective than the oxidized form in reducing the stable free radical diphenyl-*p*-picrylhydrazyl and was almost as effective in this respect as alpha-tocopherol. These pioneers also demonstrated that CoQ_6H_2 was considerably more potent than the oxidized form as an inhibitor of lipid peroxidation of arachidonic acid emulsions catalyzed by hemoglobin, and that the quinol, in this assay system, was as effective as alpha-tocopherol. These data are shown in Table 4. Additional support for an antioxidative function for reduced coenzyme Q has been provided by Booth et al.[93] who have reported that the reduced form is an effective inhibitor of ascorbate-Fe^{2+}-induced peroxidation in egg yolk phosphatidylcholine liposomes. Landi et al.[94,95] have reported that a number of coenzyme Q homologues from CoQ_2 to CoQ_{10} incorporated into sonic lipid vesicles, protect the lipids against autoxidation resulting from incubation over a period of several days. Landi et al.[94,95] report that no difference in the antioxidative potency between oxidized and reduced coenzyme Q was observed in these experiments. This deviation from previous results may be related to the possibility of autoxidation of the reduced coenzyme Q during the long period of incubation in the presence of oxygen. More recently, Landi et al.[96] have reported an inhibition by coenzyme Q_3 of lipid peroxidation in phospholipid vesicles resulting from $OH^{\cdot}$ radicals formed during ultrasonic irradiation. Landi et al.[97] have reported more recently a considerably more effective antioxidative activity of the reduced form of coenzyme Q, confirming a number of such observations by other workers.

Table 4. Inhibition of Lipid Peroxidation in Arachidonic Acid Emulsions[92]

Assay Conditions	O_2 Consumption (nmol/min)	Inhibition (%)
Initial control	58	—
CoQ_6, 1 mM	36	38
CoQ_6H_2, 1 mM	14	76
alpha-tocopherol, 1 mM	17	71
Final control	58	—

Mellors and Tappel[92] also analyzed the antioxidant effects of both oxidized and reduced CoQ_6 on the light-catalzyed peroxidation of mitochondrial lipids. These results are presented in Fig. 4 and indicate that at 2 h of light treatment, CoQ_6 inhibited lipid peroxidation by 76%, CoQ_6H_2 by 95%, and alpha-tocopherol by 100%. As both coenzyme Q and alpha-tocopherol reside in the hydrophobic milieu of the inner mitochondrial membrane, both would appear to be capable of contributing to molecular homeostasis by protecting membrane components against oxidative damage. A functional interaction between coenzyme Q and alpha-tocopherol with respect to the stability of biological membranes has been suggested by Fitch and Folkers.[98]

Mellors and Tappel[92] were also pioneers in demonstrating the protective effect of coenzyme Q, functionally reduced by electron transfer chain activity, on lipid peroxidation. Figure 5 shows the effect of beta-hydroxybutyrate, in the presence of antimycin, on the ability of exogenous coenzyme Q_6 to inhibit light-induced lipid peroxidation in rat liver mitochondria. Lipid peroxidation was inhibited to the greatest extent when both beta-hydroxybutyrate and coenzyme Q_6 were present suggesting a strong antioxidant effect of

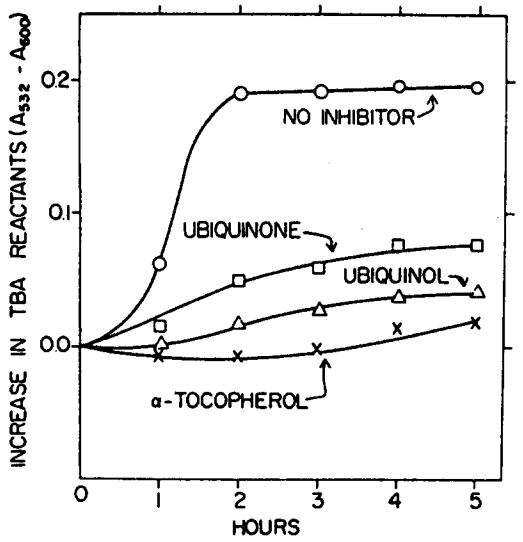

Fig. 4. Inhibition of mitochondrial photo-oxidation by exogenous coenzyme Q_6 and coenzyme Q_6H_2. Each vial contained 20 ml of a suspension of bovine liver mitochondria (2 mg of protein per milliliter of KCl-Tris, pH 7.5). Alpha-tocopherol and oxidized and reduced coenzyme Q_6 were added to 1 mM. TBA, thiobarbituric acid. [From[92] with permission.]

reduced coenzyme Q. Oxidized coenzyme Q was not as effective as the reduced quinone with respect to antioxidative activity.

Takeshige et al.[99] extended these observations to the submitochondrial level. They studied lipid peroxidation in particles from which coenzyme Q had been

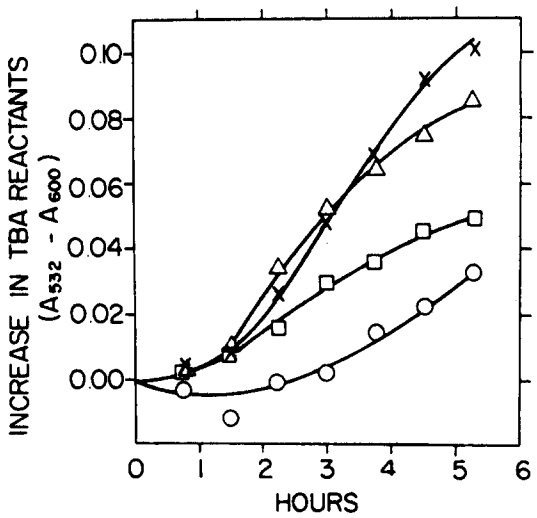

Fig. 5. Inhibition of photooxidation of rat liver mitochondria by coenzyme Q_6H_2 formed by enzymatic reduction of exogenous coenzyme Q_6. The reaction mixture contained 2 mg of coenzyme Q_6 in 0.02 ml of ethanol, 25 mM sodium beta-hydroxybutyrate, rat liver mitochondria (68 mg protein), 20 μg of antimycin in 0.02 ml of ethanol, and KCl-Tris, pH 7.5 to make a volume of 20 ml: (○), photooxidation of complete mixture; (X) no coenzyme Q_6; (□) no beta-hydroxybutyrate; (△) no coenzyme Q_6, no beta-hydroxybutyrate; TBA, thiobarbituric acid. [From[92] with permission.]

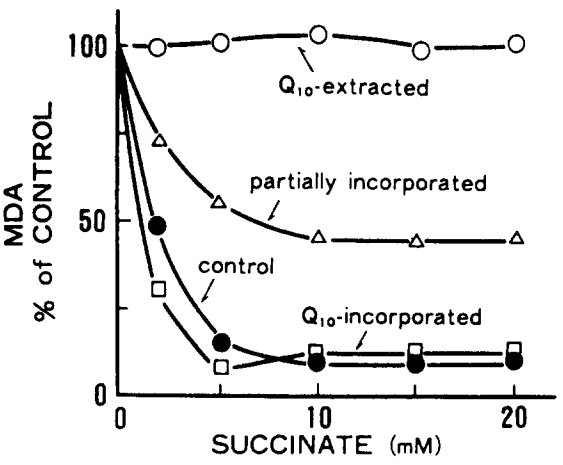

Fig. 6. Effect of coenzyme Q_{10} extraction and reincorporation on the inhibitory action of succinate on lipid peroxidation. MDA formation was assayed at pH 7.4 using rotenone. The content of enzymatically reducible CoQ_{10} in nmol/mg protein was 0.03 (○), 1.43 (△), 5.12 (□), and 3.6 (●). [From [99] with permission.]

virtually totally extracted and then reincorporated to varying known degrees.[18,99] Figure 6 demonstrates the effect of several different submitochondrial membrane coenzyme Q_{10} concentrations on lipid peroxidation during the oxidation of succinate. Reincorporation of coenzyme Q into extracted submitochondrial particles resulted in inhibition of lipid peroxidation and, in addition, the inhibitory effect of succinate was in direct proportion to the degree of reincorporation of coenzyme Q_{10}. This relationship, also shown in Fig. 6, confirms the more potent antiperoxidant effect of the reduced state of coenzyme Q.

Takeshige et al[99] have interpreted these observations on the relationship between the rate of electron transport, the formation of superoxide radical, the redox state of coenzyme Q, and lipid peroxidation as depicted in Fig. 7. Under conditions favoring coenzyme Q reduction, the rate of lipid peroxidation is low. Succinate decreases the rotenone-stimulated rate of lipid peroxidation by reducing coenzyme Q (Fig. 7, upper portion). At high concentrations of NADPH or low concentrations of NADH (Fig. 7, lower portion), the rate of electron flow is slow and electron transfer chain components, including coenzyme Q, tend to be in the oxidized state. Under such conditions, succinate oxidation provides electrons which reduce coenzyme Q and consequently inhibit lipid peroxidation.

Takeshige et al.[99] have provided additional evidence for an antioxidant role for reduced coenzyme Q. They prepared liposomes of mitochondrial lipids into which were incorporated several concentrations of coenzyme Q. The liposomes were incubated with submitochondrial particles, NADH, and rotenone, a condition previously shown to support lipid peroxidation as seen in the upper curve of Fig. 8. In the presence of succinate,

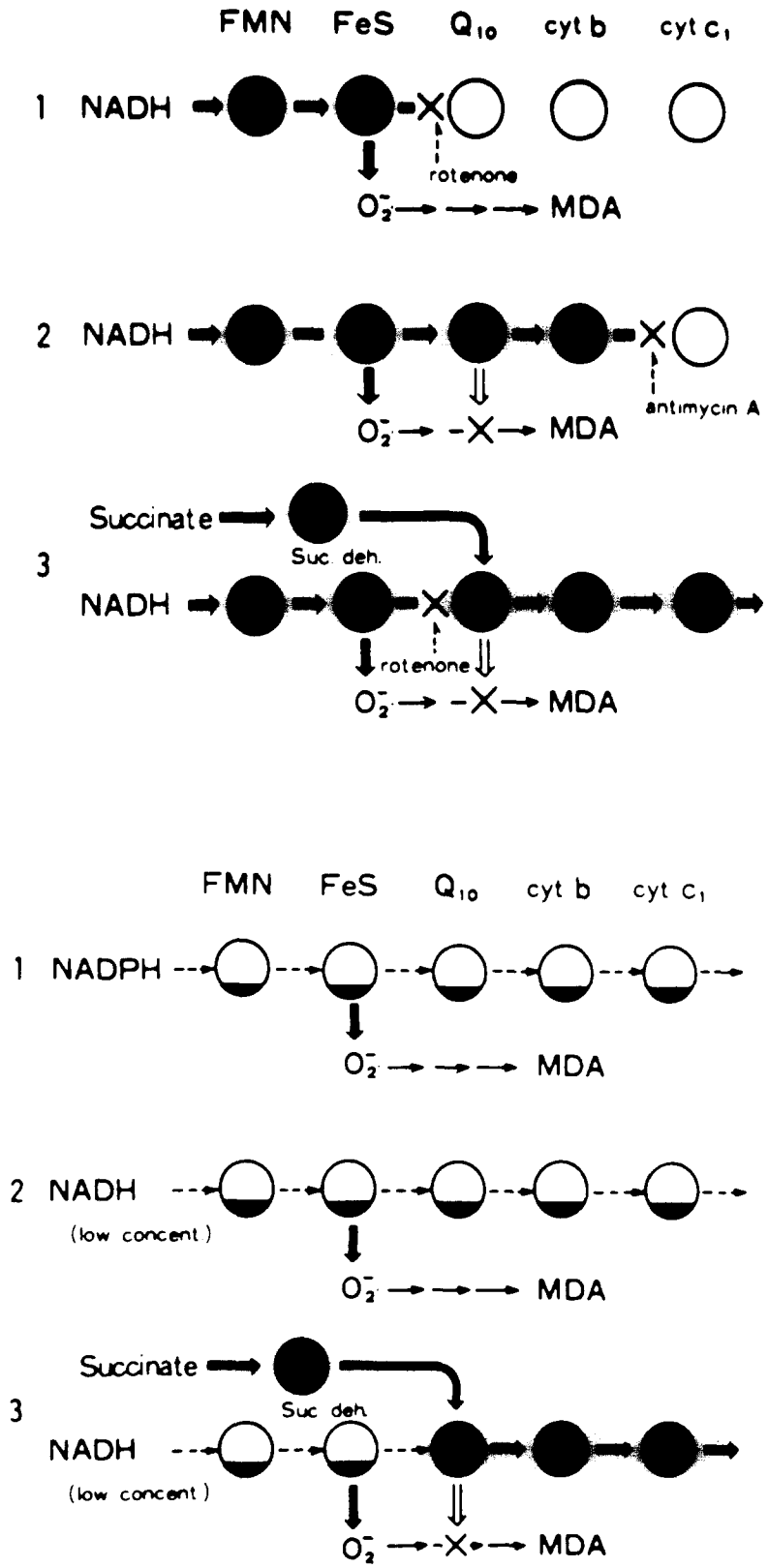

Fig. 7. The role of the redox state of endogenous coenzyme Q on free radical formation. Upper figure: MDA formation at a high rate of electron input from high levels of NADH. Lower figure: MDA formation at low rates of electron input from NADH or NADPH. [From[99] with permission.]

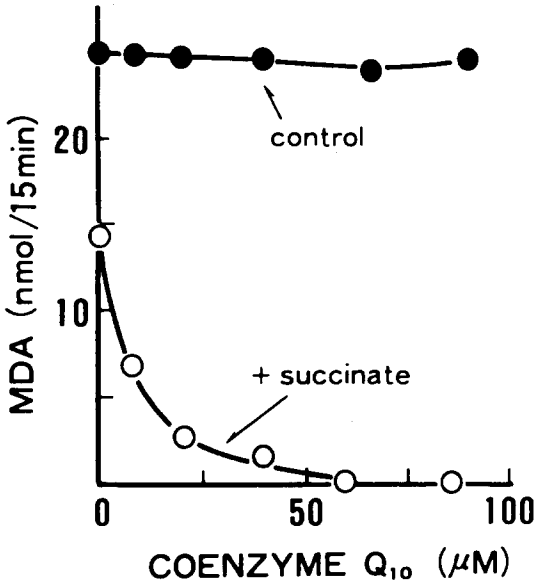

Fig. 8. Influence of the redox state of submitochondrial electron transfer chain components on lipid peroxidation in liposomes. Experimental conditions as in Fig. 6. (●), SMP (0.3 mg) and mitochondrial lipid (0.35 mg) which contains CoQ_{10} in various concentrations; (○), with 15 mM succinate. [From[99] with permission.]

lipid peroxidation was inhibited in direct proportion to the concentration of coenzyme Q, presumably in the reduced state, in the liposomes. This observation is shown in the lower curve of Fig. 8.

In addition to its effect on mitochondrial membrane systems, coenzyme Q has been shown to interfere with lipid peroxidation catalyzed by isolated rat liver microsomes.[137,38,101] The results of an experiment in which coenzyme Q_{10} in the fully reduced state was added to microsomes in which lipid peroxidation was induced[102] by NADPH and ADP-Fe^{2+} are shown in Table 5. Coenzyme Q in the oxidized form had no antioxidant effect in the particular experiment shown in Table 5, although a weak antioxidant effect was observed occasionally. The presence of oxidized coenzyme Q did not interfere with the antioxidative activity of the reduced form. Inhibition of oxygen consumption and MDA formation by $CoQ_{10}H_2$ over the range 15 to 150 μM gave an apparent K_i or I_{50} of 57 μM. The I_{50} values for two other antioxidant quinones, menadione and vitamin K_5, were also determined: 20 nM for menadione and 37 nM for vitamin K_5. This I_{50} value obtained for menadione is slightly lower than, but in good agreement with, the 37 nM reported by Talcott et al.[103]

Additional evidence in support of an antioxidant role for coenzyme Q has been derived from the demonstration[101] of the regulation by coenzyme Q of lipid peroxidation catalyzed by microsomal and mitochondrial systems isolated from carbon tetrachloride- and ethanol-treated rats. In this case the coenzyme Q was either administered to, or its concentration physiologically manipulated in, intact rats. Table 6 shows the effect of endurance training (which increases cardiac tissue coenzyme Q levels[13,35]) and age (which lowers cardiac tissue coenzyme Q levels[14,35]) on the concentration of coenzyme Q and the rate of lipid peroxidation catalyzed by submitochondrial particles isolated from hearts of rats so treated. These data indicate that cardiac inner mitochondrial membrane coenzyme Q concentration decreased 30% between 3 and 25 months of age in sedentary animals while a regimen of endurance running increased the coenzyme Q concentration of heart submitochondrial particles 41% compared to sedentary elderly animals. In addition, small differences were observed in the rate of NADH-supported lipid peroxidation catalyzed by submitochondrial particles isolated from hearts of these three groups of animals. These differences were in the direction of changes in overall respiratory activity reported previously[104-106] under these experimental circumstances. The addition of succinate to reduce endogenous coenzyme Q resulted in significant inhibition of lipid peroxidation in each case. The extent of inhibition in the presence of succinate was in direct proportion to the endogenous coenzyme Q content of the submitochondrial particles, being greatest in submitochondrial particles from 25-

Table 5. Effect of Redox State of Coenzyme Q on NADPH, ADP-Fe^{2+}-Supported Lipid Peroxidation Catalyzed by Rat Liver Microsomes[101]

		Oxygen Consumption		MDA Formed	
Additions	Concentration (μM)	(natoms/min/ mg Protein)	% of C*	(nmol)	% of C
EtOH-CL*	—	224	100	5.87	100
CoQ_{10}	150	320	143	5.71	97
$CoQ_{10}H_2$	150	59	26	1.19	20
$CoQ_{10}H_2$	300	31	14	0.26	4
CoQ_{10} + $CoQ_{10}H_2$	150 + 75	97	43	2.01	34

*Abbreviations: C, control; CL, cardiolipin.

Table 6. The Effect of Endurance Training and Age on Coenzyme Q Concentrations and Lipid Peroxidation Catalyzed by Rat Heart Submitochondrial Particles[101]

#	SMPs from	CoQ[a]	Comp[b]	P	Addn.	MDA[a]	Comp[b]	P
1	Sed-25[b]	2.4 ± .10				6.48 ± .19	2 & 1	<.001
2	Sed-25				Succinate	2.79 ± .17		
3	Tr-25	3.4 ± .10	3 & 1	<.05		7.27 ± .21	4 & 3	<.001
4	Tr-25				Succinate	1.02 ± .11		
5	Sed-3	3.6 ± .14	5 & 1	<.05		6.83 ± .18	6 & 5	<.001
6	Sed-3		5 & 3	>.5	Succinate	1.37 ± .26		

[a]nmols/mg protein.
[b]*Abbreviations:* Comp—groups compared statistically; Sed-25, Tr-25, Sed-3—25-month-old sedentary and trained, and 3-month-old sedentary rats, respectively.

month-old trained and 3-month-old sedentary rats and lowest in submitochondrial particles prepared from hearts of 25-month-old sedentary animals. It is of interest that recent reports[107,108] of myoglobin as a potential protective mechanism against oxidative injury in muscle, coupled with the finding[109] that the concentration of skeletal muscle myoglobin increases with endurance training, may provide yet another defense mechanism against oxygen toxicity in the trained muscle.

Solaini et al.,[110] employing beef heart mitochondria partially depleted of coenzyme Q by pentane extraction, have reported that lipid peroxidation induced by an adriamycin-iron complex proceeds at a higher rate than in mitochondria into which coenzyme Q has been reincorporated. Moreover, in mitochondria depleted of coenzyme Q both NADH and succinate oxidase activities were inactivated more rapidly than in mitochondria in which coenzyme Q had been replenished. These data suggest a protective role for coenzyme Q in free radical-associated inactivation of electron transfer chain function as well as membrane lipid peroxidation. Trumper et al.[111] have shown that impairment of the respiratory chain in the cytochrome b-c_1 region is an early functional event during Fe^{2+}-ascorbate induced peroxidation in rat liver mitochondria.

Cellular systems

Takahashi et al.[112] used myocardial cells in culture to test the hypothesis that coenzyme Q is able to prevent myocardial cell uptake of doxorubicin. They observed that i) coenzyme Q had no effect on the uptake of doxorubicin by the cells, ii) the intact doxorubicin molecule was taken up by the cells, and iii) treatment of the cells with coenzyme Q prior to doxorubicin administration protected the beating of the cells from doxorubicin toxicity. These observations lend further support for an antioxidative role for coenzyme Q, adding to the vast literature on the efficacy of coenzyme Q as an adjunct to quinoid chemotherapeutic agents (c.f.[113]), protecting nondividing cells from free radical damage during one-electron reduction of antitumor quinones.[114]

Intact animals and clinical studies

A number of studies on coenzyme Q function in intact animals including clinical observations have been published which, while not as incisive as experiments at the subcellular or cellular levels, are nevertheless consistent with an antioxidant function for coenzyme Q. Studies on cold-acclimated rats,[115] rats treated with thyroid hormone,[116] and other conditions also affecting the overall rate of energy metabolism of intact rats[117] revealed alterations of coenzyme Q concentrations in highly aerobic tissues. The direction of change was the same for both metabolic rate, and thus free radical production, and tissue coenzyme Q content. Significantly, the increase in tissue coenzyme Q content following thyroid hormone treatment occurred after the increase in metabolic rate,[116] suggesting that the increase in coenzyme Q may have been an adaptation to or a response to, rather than a cause of, increased oxidative activity. High in vivo oxidative activity such as that which accompanies exhaustive exercise results in a two- to three-fold increase in free radical formation and lipid peroxidation in muscle and liver.[118,119] Adaptation to endurance training reduces the susceptibility of tissue to, or provides protection from, the damaging effects of free radicals and lipid peroxidation.[120] Beyer et al.[13] have shown that cardiac and other red muscles of endurance-trained rats contain elevated levels of coenzyme Q (Table 7). Lang et al.[121] have confirmed the increase in coenzyme Q in selected tissues resulting from endurance training in the rat. Boveris et al.[122] have also reported an example of regulation of coenzyme Q concentrations independent of cytochrome c and protein of mitochondria from diabetic animals. Ogasahara et al.[123] have reported a similar phenomenon in humans. Based on these considerations, the suggestion has been made[124] that

Table 7. Coenzyme Q and Cytochrome *c* Concentration Changes with Endurance Training[13]

Muscle	pmol Cytochrome *c*/mg Protein		pmol CoQ/mg Protein	
	Sedentary	Trained	Sedentary	Trained
Heart	282	447	1078	1841
Gastrocnemius	64	124	231	457
DVL*	64	140	348	715

*Deep (red) portion of the vastus lateralis

oxyradicals may act as chemical messengers leading to the synthesis of antioxidants such as coenzyme Q. This concept has received attention recently.[125]

Further evidence consistent with an antioxidant role for coenzyme Q has been derived from analyses of coenzyme Q concentrations in tissues from rats over their life span. Beyer et al.[14] have reported that in elderly animals coenzyme Q levels drop drastically in heart, kidney, and highly aerobic (red) muscles (Table 8). This phenomenon is also depicted in Fig. 9 in which the severe decline in coenzyme Q of cardiac muscle in the aged rat is shown. These observations on aged animals are consistent with the "free radical theory of aging"[126] and with a report on an inverse correlation between longevity and peroxide-producing potential of mammalian tissues.[127] Further indications of an antioxidant role for coenzyme Q have come from studies in which the concentration of lipoperoxides in mitochondria isolated from hearts and livers of animals treated with coenzyme Q was shown to be lower than that of controls.[128] Administration of coenzyme Q to animals subjected to cardiac ischemia results in improved function of mitochondria isolated from the affected area when compared to controls.[129] Kitagiri et al.,[130] in studies of the protection from ischemic myocardial injury by intravenous administration of coenzyme Q, have shown that a portion of the administered coenzyme Q is bound to sarcoplasmic reticular membranes. In animals so treated prior to coronary artery occlusion, degradation of sarcoplasmic reticular membrane components was reduced compared to tissue from animals not treated with coenzyme Q. Shibata et al.[131] have shown that in experimental myocardial ischemia, the increase of 12-hydroxyeicosatetraenoic acid, a metabolite of arachidonic acid resulting from the action of lipoxygenase associated with injury of sarcoplasmic reticulum as well as mitochondria, is inhibited in animals previously treated with coenzyme Q. Kowasaki et al.[132] have demonstrated that administration of coenzyme Q 1 h prior to initiation of liver ischemia protects this organ from oxidative cellular damage during ischemia and reperfusion. Of particular interest is their finding that the large decrease of glutathione, alpha-tocopherol, and coenzyme Q, including reduced coenzyme Q_{10}, observed in control animals, was prevented by prior administration of coenzyme Q_{10}, suggesting a role for coenzyme Q in maintaining the efficacy of general intracellular antioxidant function. These same authors report[132] a protective role of coenzyme Q in experimental endotoxemia persuant to administration of lipopolysaccharide prepared from *E. coli*. Also noteworthy is the demonstration of a role for administered coenzyme Q_{10} in the maintenance of the reduced forms of coenzyme Q and vitamin E in the protection of the endotoxemic mouse liver.[132]

Table 8. Coenzyme Q Concentration Changes with Age[14]*

Tissue	Age (months)		
	2	18	25
Heart	1134	2036	825
Kidney	501	883	688
Gastrocnemius	210	340	233
DVL†	365	522	408
Soleus	364	338	240

*Coenzyme Q concentrations: nmols/mg protein.
†Deep (red) portion of the vastus lateralis muscle.

The administration of coenzyme Q partially reverses the severe decline of respiratory metabolism of cardiac tissue which occurs in animals of advanced age.[35] This effect is most striking with the oxidation of long-chain fatty acids, the substrate of choice in cardiac muscle. Further evidence for an antioxidant effect of coenzyme Q in intact animals has been offered by Quinn et al.[133] who report that coenzyme Q administration protects liver cells against oxidative damage resulting from exposure to carbon tetrachloride. Bertelli et al.[134] have confirmed these observations. Employing either carbon tetrachloride or ethanol to produce profound and moderate free radical damage in intact rats respectively, Beyer[101] has reported that the administration of coenzyme Q results in an inhibition of CCl_4- and ethanol-stimulated lipid peroxidation using urinary malondialdehyde as an index (Table 9). In the same study,[101] coenzyme Q_{10} administration was shown to lower malondialdehyde levels in liver homogenate, mitochondrial and microsomal fractions from both CCl_4- and ethanol-treated rats (Table 10). As it is well established that administration of carbon tetrachloride[135] and ethanol[136,137] results in free radical damage, these data are consistent with an antioxidative function for coenzyme Q in vivo.

In a study of the influence of in vitro coenzyme Q antagonists on doxorubicin toxicity in vivo, Tabora et

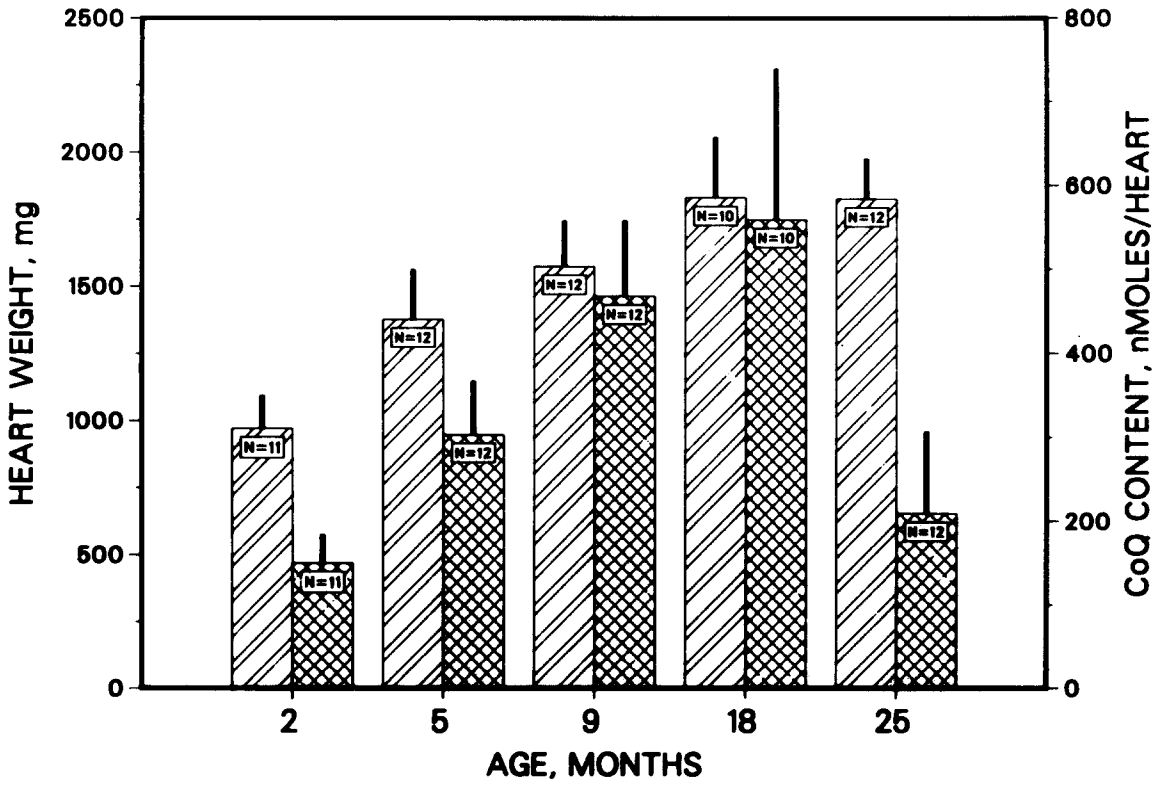

Fig. 9. Age versus heart weight and coenzyme Q content in the Sprague–Dawley rat. [From [14] with permission.]

al.[138] report toxicity by such antagonists, an effect completely blocked by coenzyme Q pretreatment, but only reduced by alpha-tocopherol pretreatment. Coenzyme Q antagonists enhanced doxorubicin toxicity, an interactive effect not diminished by coenzyme Q pretreatment. As reduced coenzyme Q is the most effective antioxidant form of the compound, the inability of coenzyme Q to prevent doxorubicin toxicity in the presence of antagonists may be due to interference with the ability of the electron transfer chain to reduce coenzyme Q in the presence of the antagonists.

In addition to the vast literature on successful applications of coenzyme Q in a variety of clinical situations,[139–145] recent findings of clinical significance have increased the medical community's interest in coenzyme Q, especially in the areas of congestive heart failure and certain neurologic disorders. Karlsson et al.[146] have reported that patients under treatment with adriamycin have lower coenzyme Q levels compared to normal controls in heart and skeletal muscle as well as in venous blood; coenzyme Q is essentially depleted in skeletal muscle of several patients. In addition, another study of coenzyme Q and congestive heart failure[147] reports that upon withdrawal of long-term coenzyme Q_{10} therapy, clinical decline, relapse, and death occurred within 2 weeks in two subjects. Several patients who relapsed after coenzyme Q withdrawal regained previous functional status after retreatment with coenzyme Q.

Kearns–Sayre syndrome is a mitochondrial encephalomyopathy characterized by the triad of progressive external ophthalmoparesis, heart conduction

Table 9. TBA-reacting Material in 24 h Urine Samples of Rats Treated with CCl_4, Ethanol, and Coenzyme Q[101]

Group #	Treatment	n	Urine MDA (μg/24 h)	Groups Compared	P
1	Control	6	0.92 ± 0.045		
2	CoQ	6	0.62 ± 0.037	2 vs 1	NS
3	CCl_4	5	4.88 ± 0.32	3 vs 1	<0.01
4	CCl_4 + CoQ	6	1.46 ± 0.18	4 vs 3	<0.01
5	EtOH	7	2.42 ± 0.22	5 vs 1	<0.05
6	EtOH + CoQ	6	0.81 ± 0.07	6 vs 5	<0.05

Data as Mean ± SE.*

Table 10. TBA-reacting Material (MDA) in Liver Fractions from Rats Treated with CCl_4, Ethanol, and Coenzyme Q[101]

GP #	Treatment	n	Liver Fraction								
			Homog[a]	Comp[c]	P	Mito[b]	Comp[c]	P	Mic[b]	Comp[c]	P
1	Control	8	86 ± 4	—	—	8.2 ± 0.3	—	—	5.4 ± 0.3	—	—
2	CoQ	6	13 ± 1	2 vs 1	<.001	3.7 ± 0.3	2 vs 1	<.01	3.2 ± 0.1	2 vs 1	<.02
3	CCl_4	8	766 ± 14	3 vs 1	<.001	28.3 ± 2.8	3 vs 1	<.05	10.7 ± 0.8	3 vs 1	<.05
4	CCl_4 + CoQ	7	465 ± 13	4 vs 3	<.001	11.1 ± 0.7	4 vs 3	<.05	6.5 ± 0.4	4 vs 3	<.1
5	EtOH	6	188 ± 7	5 vs 1	<.001	19.8 ± 2.2	5 vs 1	<.05	8.4 ± 0.4	5 vs 1	<.05
6	EtOH + CoQ	5	15 ± 2	6 vs 5	<.001	4.6 ± 0.4	6 vs 5	<.02	3.4 ± 0.5	6 vs 5	<.01

[a]nmol MDA/gram liver; [b]nmol MDA/mg protein; [c]Comp, Groups compared statistically.
Abbreviations: Homog = homogenate, Mito = mitochondrial, Mic = microsomal.
*Data as Mean ± SE.

block, and pigmentary degeneration of the retina, which commence in childhood. Such patients show reduced levels of coenzyme Q_{10} in serum and mitochondria isolated from skeletal muscle and respond favorably to coenzyme Q_{10} therapy.[123,148-151] Zierz et al.[152] have confirmed these observations in patients with Kearns–Sayre syndrome and ophthalmoplegia. In a recent publication, Ogasahara et al.[123] report a case of familial mitochondrial encephalomyopathy associated with progressive muscle weakness, abnormal fatigability, and central nervous system dysfunction since childhood. State 3 respiratory rate of muscle mitochondria with NADH-linked substrates and with succinate was markedly reduced. The levels of cytochromes $a + a_3$, b, and $c + c_1$ were normal as were the activities of individual complexes I, II, III, and IV of the electron transport chain. By contrast, the activities of complexes I–III and II–III, both of which require coenzyme Q_{10} for activity, were abnormally low. The mitochondrial coenzyme Q_{10} content was found to be 3.7% of that found in 10 controls while serum coenzyme Q levels were normal. These and previous findings i) strengthen the possibility that discrete pathology may result from reduced tissue levels of coenzyme Q, ii) provide evidence for the concept of tissue specific defects in coenzyme Q biosynthesis, and iii) enforce the rationale for the treatment of such clinical entities with coenzyme Q. Fischer et al.[153] were the first to report a defect at the level of coenzyme Q in a case of mitochondrial encephalomyopathy.

THEORETICAL DISCUSSION: MECHANISMS

The mechanism by which reduced coenzyme Q acts as an antioxidant remains unresolved. Several possibilities exist, however. Reduced coenzyme Q could react with ADP-perferryl ions as suggested by Svingen et al.[154]:

$$ADP\text{-}Fe^{3+}\text{-}O_2^{\cdot-} + CoQH_2 \longrightarrow ADP\text{-}Fe^{2+} + H_2O_2 + CoQ \quad (8)$$

H_2O_2 would be removed by catalase, peroxidase, or glutathione peroxidase. Alternatively, reduced coenzyme Q could react with superoxide directly as a free radical quencher,

$$2O_2^{\cdot-} + CoQH_2 \longrightarrow H_2O_2 + O_2 + CoQ \quad (9)$$

and thus interfere with the initiation of lipid peroxidation. Reduced coenzyme Q might also react with lipid free radicals or lipid peroxide free radicals,

$$2L^{\cdot} + CoQH_2 \longrightarrow 2LH + CoQ \quad (10)$$

$$2LOO^{\cdot} + CoQH_2 \longrightarrow 2LOOH + CoQ \quad (11)$$

and therefore prevent the propagation reaction of lipid peroxidation. As Landi et al.[95] have shown that CoQ_3H_2 and CoQ_9H_2 are equally effective as antioxidants in sonic phospholipid vesicles, the length of the polyisoprenoid chain of CoQ would not appear to be a determining factor in the mechanism of antioxidation by this compound.

The question also arises as to whether all of the coenzyme Q in the membrane is available to function as an antioxidant or whether a special coenzyme Q pool, or compartment, exists with a unique antioxidant role. The data of Kroger and Klingenberg[19] suggest that coenzyme Q functions as a homogeneous pool during electron transport. More recent evidence[155,156] indicates that three distinct coenzyme Q pools exist in pigeon heart mitochondria. One of the three pools is relatively inactive metabolically and consists of both oxidized and reduced coenzyme Q.[155,156] It is possible that such a pool, in slow equilibrium with the electron transfer chain, may be involved in an antioxidant role. In this regard, it is of interest that the mitochondrial concentration of coenzyme Q may be lowered by 50% following treatment of neuroblastoma cells in culture with mevinolin, an inhibitor of 3-hydroxy-3-methylglutaryl CoA reductase required for the synthesis of the isoprenoid chain of coenzyme Q, without affecting

the respiratory function of the mitochondria.[157] As the lowering of the coenzyme Q concentration coincides with inhibition of cell cycling,[157] it is tempting to speculate that the portion of the coenzyme Q pool which is lowered in concentration is concerned with protection against oxidative damage and that its loss results in an inability to proliferate. It is also possible that a failure to synthesize sufficient dolichols required for the glycosylation reaction in the formation of glycoproteins is involved in this loss of proliferative capacity. Carson and Lannarz [158] have shown that compactin, which is also a potent inhibitor of hydroxymethylglutaryl-CoA reductase required for the synthesis of cholesterol and dolichol as well as coenzyme Q, interferes with embryonic development and that the inclusion of exogenous dolichol allows normal development to proceed.

The apoprotein-bound form of coenzyme Q in mitochondrial electron transfer chain complexes I, II, and III may be positioned to kinetically favor the rapid transfer of electrons to the cytochrome system during state 3 respiration. During state 4, apoprotein-bound reduced coenzyme Q may then transfer reducing equivalents to an "antioxidant coenzyme Q pool." The position of oxidized coenzyme Q is restricted in the inner mitochondrial membrane to a domain where it does not perturb the phase transition of the phospholipids, while its reduction, resulting in an increase in hydrophilicity, allows for greater mobility throughout the hydrocarbon phase of the mitochondrial membrane.[159] Such freedom of movement may be of significance in enhancing the antioxidant role of an "antioxidant coenzyme Q pool" by apoprotein-bound coenzyme Q during state 4 respiration.

In addition to the elimination of preformed oxygen radicals by way of superoxide dismutase, catalase, various peroxidases, and other antioxidant factors, there exists a mechanism by which superoxide anion formation can be prevented. It involves the enzyme DT diaphorase[160] which catalyzes a two-electron transfer[161] from NADH or NADPH to quinones to produce the relatively stable quinol, thus preventing semiquinone formation and subsequent free radical production.[162] Because of its ability to protect against carcinogen-induced cancer,[163] DT diaphorase has been referred to as an anticancer enzyme[164] and "a potential cancer protecting enzyme."[165] In the cytosol, which contains the bulk of DT diaphorase activity, the enzyme most likely serves as a device for the detoxification of exogenous quinones.[162] Microsomal DT diaphorase has been shown to serve as a vitamin K reductase[166] required for the synthesis of prothrombin and related proteins. In mitochondria, which also contain a minor portion of cellular DT diaphorase,[167] the role of the enzyme is not known, but may be related to quinone reduction as well. It is conceivable that mitochondrial DT diaphorase interacts with coenzyme Q and participates in regulation of its redox state, thus influencing its antioxidant function. The results of preliminary experiments from this laboratory involving phospholipid micelles containing DT diaphorase and coenzyme Q suggest that some such interaction in the hydrophobic milieu may take place. Dallner has reported recently[168] that a portion of the coenzyme Q found in the cytosolic fraction is bound to DT diaphorase.

Another possible mechanism to explain the antioxidant capacity of $CoQH_2$ is derived from the observation of Cadenas et al.[169] that superoxide dismutase may interact with various hydroquinones and, in conjunction with the action of DT diaphorase, inhibit autoxidation of hydroquinones. They propose a new activity for superoxide dismutase, an $O_2^{\cdot-}$-semiquinone oxidoreductase, in which SOD-Cu^{++} would be reduced by $O_2^{\cdot-}$,

$$\text{SOD-Cu}^{++} + O_2^{\cdot-} \longrightarrow \text{SOD-Cu}^{+} + O_2, \quad (12)$$

followed by oxidation of SOD-Cu^+ by a semiquinone intermediate,

$$\text{SOD-Cu}^{+} + Q^{\cdot-} \longrightarrow \text{SOD-Cu}^{++} + Q^{2-}. \quad (13)$$

If, in fact, DT diaphorase is able to convert CoQ to CoQ^{2-} or $CoQH_2$, the sum of the two reactions above would be,

$$O_2^{\cdot-} + CoQ^{\cdot-} \longrightarrow O_2 + CoQ^{2-} \quad (15)$$

and would serve as a mechanism to dismutate the coenzyme Q semiquinone to the more stable hydroquinone and interfere with the transfer of the unpaired electron of the coenzyme Q semiquinone to oxygen to form the superoxide radical. The role of DT diaphorase in redox and addition chemistry of quinoid compounds and the biological implications have been reviewed recently.[170]

The question of the means by which coenzyme Q administration results in the myriad of clinical and experimental benefits reported in several volumes[139-145] remains essentially unanswered. Whether such administration restores coenzyme Q to a level required to maintain an adequate rate of energy release from electron transfer chain reactions and energy conservation during oxidative phosphorylation or, alternately, provides coenzyme Q as an antioxidant to protect tissue at jeopardy from serious peroxidative damage, or both, will require further incisive research. Nevertheless, it would appear that sufficient evidence exists to allow the assignment of an antioxidant role to coenzyme Q

in addition to its other well-established cellular functions. While the participation of coenzyme Q in free radical formation in the cell under physiological conditions may be questionable, the ability of a number of other quinone species to induce oxidative injury or act as antioxidants[171] should be noted. It would appear that coenzyme Q, based on its wide, albeit not ubiquitous, distribution in biological systems and its several functions, has been the subject of intense molecular selection during the evolution of aerobic species.[38] The presence of coenzyme Q in mitochondrial, lysosomal, Golgi, plasma, peroxisomal, and microsomal membranes[172] may be interpreted as an indication of a wider antioxidant function for coenzyme Q than heretofore considered. It is also of interest that the majority of tissue coenzyme Q molecules exist in the reduced form.[168]

FUTURE DIRECTIONS FOR COENZYME Q RESEARCH

As suggested above, a number of aspects of coenzyme Q function remain open to investigation. The discovery of functional coenzyme Q-binding proteins, or apoproteins, opens the possibility that coenzyme Q may be transported, as are other antioxidants, by carrier proteins in blood and other tissues. Although recent evidence[172] suggests that coenzyme Q is synthesized by the endoplasmic reticulum and transported to other cellular membranes,[173] the means of transport and its target specificity remain unknown and thus a challenge. Initial studies suggest that coenzyme Q may be released from the liver and transported to bile in combination with lipoproteins.[174,175] In addition, the regulation of the turnover of coenzyme Q is pertinent from a physiological as well as a clinical perspective. Yamamoto et al.[176] and Dallner and his colleagues[173] have contributed to this area by their identification of regulatory sites in the biosynthesis of coenzyme Q. The relation between the synthesis of coenzyme Q and cholesterol, both polyisoprenoid compounds, is also of interest to the biological and clinical fields. It has been observed that oral administration of coenzyme Q increases its concentration in liver and inhibits the synthesis of cholesterol in vivo, leading to the hypothesis that hypocholesterolemic agents exert their physiological action via coenzyme Q.[177] The plasticizer diethylhexyl phthalate has been reported to both lower blood cholesterol and to increase liver coenzyme Q levels by stimulating coenzyme Q biosynthesis.[178] Studies on the design, synthesis, and mode of action of compounds related to coenzyme Q are beginning to be published. Imada et al.[179] have reported that idebenone, a low molecular weight benzoquinone related to coenzyme Q, restores the ability of brain mitochondrial preparations, from which coenzyme Q has been extracted, to catalyze the oxidation of succinate and NADH. The NADH-cytochrome *c* reductase activity of such preparations, damaged as a result of free radical formation, is protected by idebenone.[179] Idebenone has also been shown[180] recently to be an effective adjunct to coenzyme Q_{10} therapy in cases of mitochondrial encephalomyopathy. In addition, future clinical studies on coenzyme Q will have to incorporate observations on the toxicity and mutagenicity of coenzyme Q and its metabolites, regardless of the lack of reported side effects in its clinical use to date. Recent studies have provided evidence that the oral,[181] intravenous,[182] and topical[183] routes of coenzyme Q administration are all effective in raising tissue coenzyme Q concentrations.

The clinical adjunct use of coenzyme Q to interfere with adverse side effects resulting from the use of other drugs is expanding. Glaucoma, predominantly a condition of the elderly, is treated with beta-blockers which frequently exacerbate preexisting cardiovascular conditions. Takahashi et al.[184] have reported recently that oral administration of coenzyme Q_{10} to such glaucoma patients is useful in mitigating cardiovascular side effects without interfering with the lowering of intraocular pressure by beta-blockers.

A FOOTNOTE

As a footnote, I would like to offer the opinion that molecules such as coenzyme Q have been selected during evolution to perform primary chemical roles related to their qualitative chemical reactivity. Some of these molecules may perform secondary functions as a result of incidental reactions related to their primary function or may perform incidental biological roles as a result of possessing molecular characteristics unrelated to their primary function. For example, the primary and only known function of alpha-tocopherol is to act as an intramembrane antioxidant.[185,186] On the other hand, coenzyme Q functions as an electron transporter in the mitochondrial electron transfer chain and in the Q-cycle of energy conservation (its primary functions) and, in addition, as an antioxidant when in the hydroquinone form (its secondary or incidental function). A number of other molecules such as myoglobin,[107,108,187] bilirubin,[188] taurine,[189] uric acid,[190] chlorophyll,[191] and others have been shown to possess unrelated specific primary and secondary antioxidant functions. This concept does not diminish the value or importance of the incidental antioxidant function of such molecules to the proper functioning of the organism. It is intended to provide an accurate pedagogic view of their biological roles.

SUMMARY

Evidence in support of the role for coenzyme Q in free radical production and as an antioxidant has been reviewed. Although the formation and existence of the semiquinone form of coenzyme Q has been demonstrated in vitro, the metabolic conditions required for its formation may be difficult to achieve in the intact animal. In addition, a good deal of the literature suggesting a role for coenzyme Q in free radical formation relies on indirect evidence and does not exclude the participation of other chemical species such as flavin and iron-sulfur functions in free radical production.

The many demonstrations that coenzyme Q may act as an antioxidant at many levels of biological organization, including the clinical setting, provide strong support for the concept that coenzyme Q was selected during evolution of aerobic species to protect the several types of membranes in which it resides from free radical damage. Although the chemical mechanism(s) whereby coenzyme Q exerts its protective effects has not been elucidated, future research in this area should be fruitful. Thus, consideration must be given to the view that coenzyme Q has hormetic properties. Under some circumstances it may contribute to free radical formation and thus have toxic effects on the cell whereas under other conditions it would appear to provide protection from just such damage. Albert Szent-Gyorgyi envisioned the protective role of hydroquinones from free radical damage in the 1950s and urged his friends to eat wheat germ, containing methoxyhydroquinone and other quinones, daily.[192]

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ABBREVIATIONS

CoQ—coenzyme Q
$CoQH_2$—reduced coenzyme Q
$CoQ^{\cdot-}$—coenzyme Q semiquinone
MDA—malondialdehyde
SOD—superoxide dismutase
TBA—thiobarbituric acid
SMPs—submitochondrial particles