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IS REDOX-CYCLING UBIQUINONE INVOLVED IN MITOCHONDRIAL OXYGEN ACTIVATION?

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In most tissues mitochondria consume more than 90% of cellular oxygen. Although the greatest part of it undergoes tetravalent reduction thereby conserving free energy changes in the form of ATP, a great deal of evidence exists in the literature that also univalently reduced dioxygen is released during respiration. Redox-cycling ubiquinone was considered most frequently to be involved in this univalent e^- transfer to oxygen out of sequence however, other components of the respiratory chain could not be excluded. Our investigations on this problem questioned the role of redox-cycling ubiquinone in mitochondrial $O_2^{\cdot-}$ formation while H_2O_2 is supposed to accept e^- from this source. The paper provides experimental evidence that H_2O_2 in fact may operate as an oxidant of ubisemiquinone while dioxygen requires protons for such a reaction which are not available in the phospholipid bilayer where ubiquinone undergoes one e^- redox-cycling.

KEY WORDS: Mitochondria, ubisemiquinone, cytochrome b, superoxide radicals, hydroxyl radicals.

INTRODUCTION

The detection that under certain conditions of respiration mitochondria release small amounts of $O_2^{\cdot-}$- and $OH^{\cdot}$-radicals[1-6] indicates the possibility of a deviation of electrons from their normal pathway in the respiratory chain both to dioxygen and H_2O_2. Redox-cycling ubiquinone (*UQ*) was most frequently considered to play a role in single e^--transfer to oxygen out of sequence.[1,7-9] The following functional properties of *UQ* in mitochondrial electron transfer strongly support this concept. Reduction and oxidation of *UQ* which mediates electron flux from dehydrogenases of respiratory substrates to cytochromes is accomplished by successive one electron redox-steps and involves free moving of this electron carrier between its acceptor and donor sites. These peculiarities of redox-cycling *UQ* make it susceptible to appropriate non-mitochondrial electron acceptors which may undergo one-electron reduction.

In line with this Trumpower and Simmons have concluded from their observation of a SOD-sensitive reduction of cyt c in the presence of antimycin (AA) and thenoyltrifluoroacetone (TTFA) that apart from regular oxidants of the respiratory chain oxygen also may serve as an oxidant of one-electron cycling ubisemiquinone at center out (SQ_0). The unpaired electron of SQ_0 is then further transferred to cyt c via a short circuit.[9]

Although most of the investigators in this field could not directly measure the existence of redox-cycling SQ_0 due to its low stability constant, there is convincing indirect evidence that electrons will deviate from the ubiquinone-loop (for illustration see scheme of Figure 1) to molecular oxygen under conditions where SQ_0 was supposed to exist in high steady state concentrations. This was the case in tightly

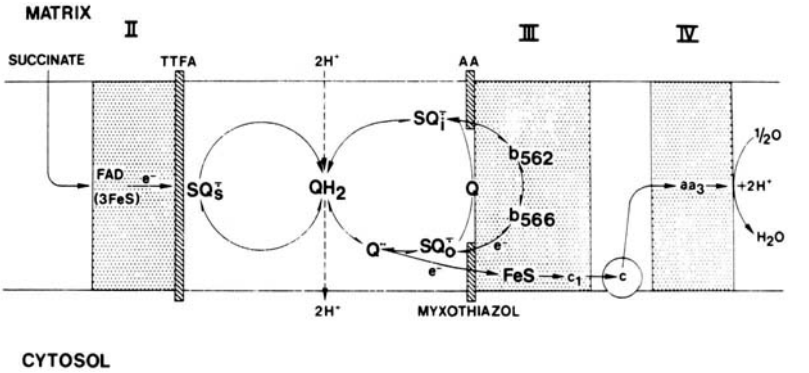

FIGURE 1 Scheme of ubiquinone-mediated e^- transfer between succinate dehydrogenase of complex II and the bc_1 complex (complex III). Electrons from complex II are carried through the ubiquinol-pool (QH_2) and the first e^- is linearly transferred to the Rieske iron sulfor protein (FeS) following binding close to the outer phase of the inner mitochondrial membrane at complex III. The second e^- of ubisemiquinone formed at center out (SQ_0) is then recycled into the QH_2-pool after equilibration with b-type-cytochromes completing a loop of e^--cycling (UQ-loop). Associated with the linear transfer of one electron two protons are extruted from the matrix to the cytosol.

TABLE I

CONDITIONS	OXYGEN ACTIV. Mito	OXYGEN ACTIV. SMP	REDOX-CYCL. UQ	CYT b REDUCED	REFERENCES
UQ removal/ reconst.	H_2O_2	–	+	+	Boveris & Chance, 1973 (1)
Antimycin	H_2O_2	$O_2^{\cdot}$	+	+	Loschen et al, 1973 (2)
Antimycin	H_2O_2	$O_2^{\cdot}$	+	+	Nohl & Hegner, 1978 (4)
state IV respiration	H_2O_2	–	+	+	Loschen et al, 1973 (18)
ATP-induced rev. e^- flow	H_2O_2 $O_2^{\cdot}$	–	+	+	Nohl & Hegner, 1978 (4)
TTFA + Antimycin	$O_2^{\cdot}$	–	$(UQ_0^{\cdot})^*$	+	Trumpower & Simmons, 1979 (9)
TCDD	H_2O_2 $O_2^{\cdot}$	$O_2^{\cdot}$	+	+	Nohl et al, 1989 (19)
Fum/Succ. poise 0 mV	–	–	$(UQ_S^{\cdot})$	–	Nohl et al, 1982, 1986 (6,5)
Myxothiazol	–	–	–	–	Turrens & Lehninger, 1985 (20)
Myxothiazol	–	–	$(UQ_S^{\cdot})$	–	Nohl & Jordan, 1986 (5)

* not directly measured

coupled mitochondria respiring under state IV conditions, in the presence of AA and under conditions of ATP induced reverse-electron flow. Table I summarizes data from various laboratories which all stress ubisemiquinone to be the site where electrons leak out of the loop to dioxygen under conditions of $O_2^{\cdot-}$ release. However, under all these conditions also b-type cytochromes were found to exhibit a high state of reduction (see Table I).

Since the establishment of an efficient redox-couple requires a permanent shuttle of reducing equivalents to the respective reductant both, redox-cycling ubiquinone and b-type cytochromes may be considered to operate as an electron donor for molecular oxygen under conditions mentioned above.

RESULTS AND DISCUSSION

An experimental approach to evaluate which of these electron carriers is involved we have correlated changes of the redox-states both of b-type cytochromes and redox-cycling *UQ* with the release of $O_2^{\cdot-}$ radicals from mitochondria.

Figure 2 Aa shows the classical experiment of $O_2^{\cdot-}$ formation from SOD-free submitochondrial particles (SMP) in the presence of AA.[3] The inhibitory effect of SOD on the cooxidation of epinephrine to adrenochrome clearly demonstrates that $O_2^{\cdot-}$ have been formed after the addition of AA to succinate respiring SMP. Since AA prevent recycling of electrons from b type cytochromes into the *UQ*-loop (see Figure 1) both high and low potential cyt b equilibrate in the reduced state.[10] This is reflected by a rapid and extensive extra-reduction of cyt b_{566} as shown in Figure 2 Ba. Myxothiazole (myx) a compound reported to prevent recycling of electrons into the *UQ*-loop at the level of SQ_0[11] (see Figure 1) also inhibits AA-stimulated $O_2^{\cdot-}$ release from SMP (Figure 2 Ab).

Although cyt b_{566} exhibited a small increase in the reduction state the kinetic trace of myx induced reduction is clearly different from the one observed in the presence of AA elucidating the opposing effects of the two inhibitors on the redox steady state of low potential cyt b.

Myx together with AA deprive b-type cyt from electron cycling (Figure 2 Bc) and

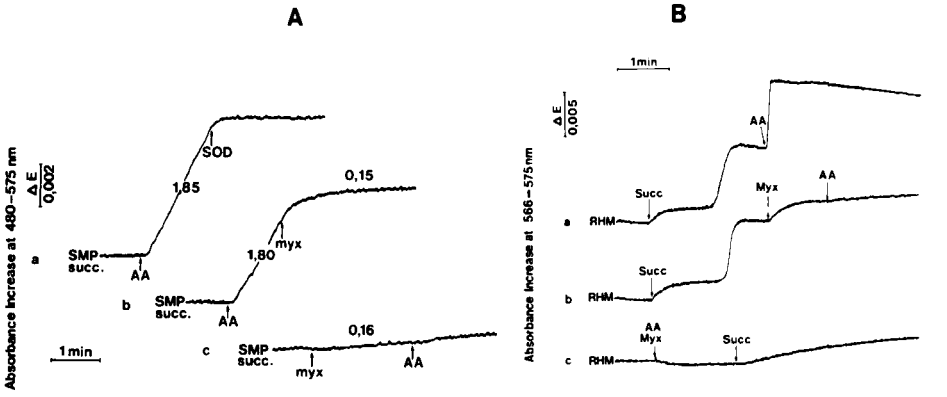

FIGURE 2 Relation between redox states of cyt b_{566} and the release of $O_2^{\cdot-}$ from SMP of rat hearts. For experimental details see ref.[5]

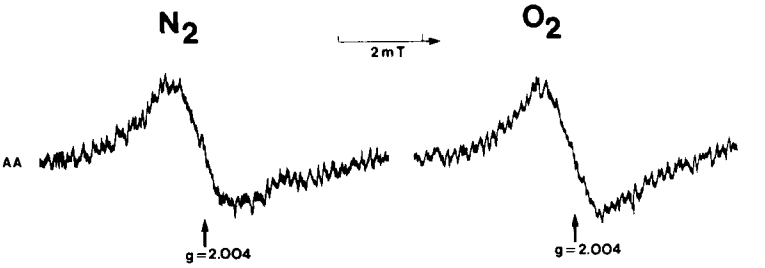

FIGURE 3 Influence of dioxygen a steady state concentrations of mitochondrial ubisemiquinones. Ubisemiquinones were identified by a Bruker ESR spectrometer B-ER 4/4S as in.[5]

also single electron transfer to molecular oxygen was found to be inhibited (Figure 2 Ac).

These observations clearly demonstrate that molecular oxygen may in fact operate as an electron acceptor of the respiratory chain at sites different from cyt-oxidase. The opposing effects of AA and myx both, on the redox states of low potential cyt b and $O_2^{\cdot-}$ formation rates suggest a role of this cytochrome species in the univalent electron transfer to O_2 out of sequence. Thermodynamically such a redox couple is conceivable, since the calculated redox potential of this couple is in favour to $O_2^{\cdot-}$ formation.

However, AA was reported to increase also steady state concentrations of redox-cycling ubiquinone at center out[9,10] while myx prevents SQ_0 formation and destabilizes SQ_0 bound to cyt b_{566}. Thus, it was of interest to investigate whether oxygen may operate as an oxidant of redox-cycling ubiquinone under conditions where mitochondria were shown to release $O_2^{\cdot-}$.

Figure 3 demonstrates that under these conditions of mitochondrial respiration SMP exhibited a stabile ubisemiquinone radical which was identified by means of the ESR technique. Although its equilibrium concentration was adjusted at a labile level by limiting electron flow rates from succinate with fumarate oxygen was without effect on steady state concentrations of ubisemiquinone.

Assuming that semiquinone pools of the *UQ* loop are kinetically in equilibrium the insensitivity of steady state concentrations questioned whether molecular oxygen may operate as an oxidant of redox-cycling ubiquinone in the phospholipid bilayer of the inner mitochondrial membrane. Thermodynamic considerations on one e^--redox-potentials of *UQ* and O_2 in aprotic systems based on the literature[13-15] also exclude the possibility of such a reaction.

To approach this question experimentally we have designed a non-protic reaction system in which the equilibrium of a reaction mixture of biological relevant quinones and superoxide radicals was followed. Figure 4 demonstrates the existence of semiquinone-related single line ESR spectra which could be obtained in all cases of quinones after the addition of an $O_2^{\cdot-}$ generator (KO_2 in crown ether).

The semiquinones remained stabile despite the presence of air oxygen while protonation of the reaction system (by the addition of small amounts of water) caused their immediate disappearance (Figure 5a,b). Simultaneously $O_2^{\cdot-}$ radicals could be detected by means of spin trapping with DMPO (Figure 5c). The corresponding ESR signal was identified by a model experiment in which $O_2^{\cdot-}$ from KO_2 were spin-trapped by DMPO in the same reaction medium (Figure 5d). These findings clearly indicate

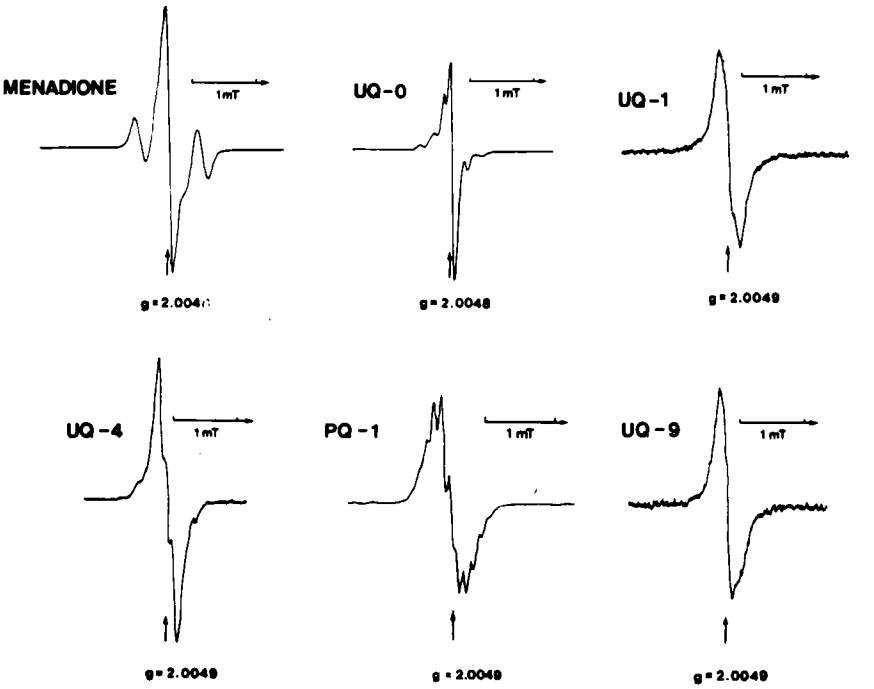

FIGURE 4 ESR-spectra of semiquinone radicals generated by monovalent electron transfer from $O_2^{\cdot-}$ in acetonitrile. Experimental details are described in.[21]

that in hydrophobic systems the equilibrium of the reaction

$$UQ + O_2^{\cdot-} \rightleftharpoons UQ^{\cdot} + O_2$$

is on the side of $UQ^{\cdot}$ formation while protonation shifts the reaction to the left.

Assuming redox-cycling ubiquinone to operate in an aprotic phase of the phospholipid bilayer[16] its semiquinone form seems unlikely to interact with molecular oxygen.

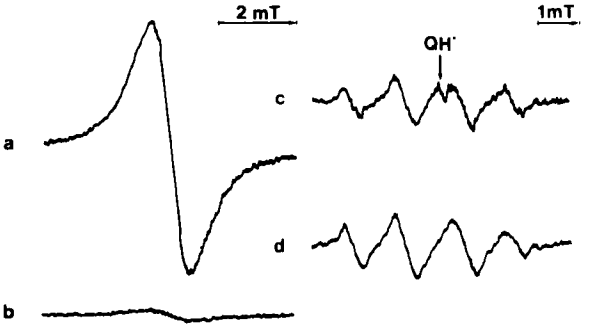

FIGURE 5 The effect of protons on the stability of ubisemiquinone-related ESR spectra in acetonitrile equilibrated with air-oxygen. Experimental details are given in the text and in.[5]

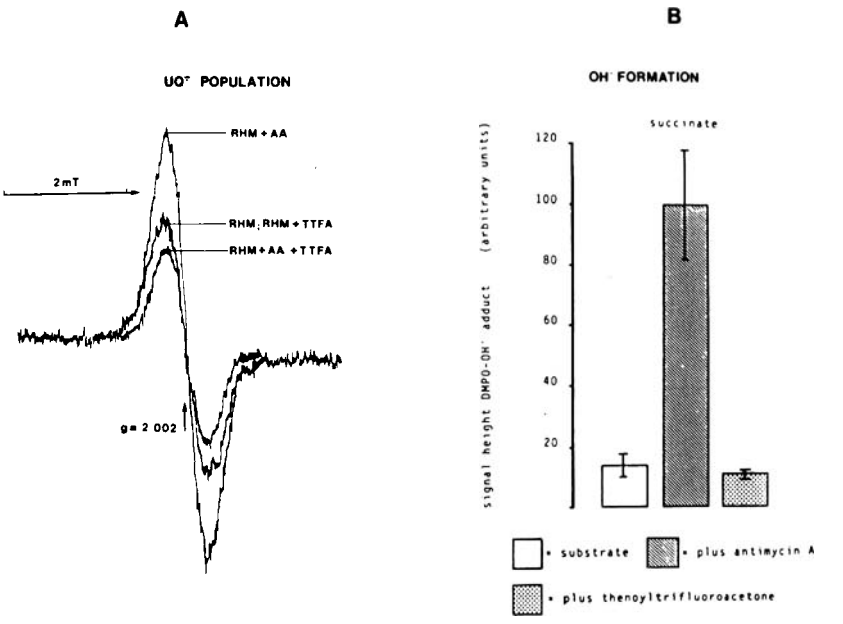

FIGURE 6 Quantitative correlation between steady state concentrations of mitochondrial ubisemiquinones and spin adduct formation of free OH· with DMPO following the addition of H_2O_2. Steady state concentrations of ubisemiquinones were varied by the addition of AA and TTFA to succinate respiring mitochondria. The experiments were carried out in a Bruker ESR-spectrometer at room temperature. More experimental details are described in the original paper.[6]

However, our observations of a quantitative relationship between steady state concentrations of mitochondrial ubisemiquinones and OH· formation from added H_2O_2 (Figure 6) suggest the unpaired e^- of redox-cycling ubiquinone can also be transferred to H_2O_2. The detection of OH· radicals in this system indicates the initiation of a reductive homolytic cleavage of H_2O_2 according to

$$UQ^{\cdot} + H_2O_2 \rightarrow UQ + OH^{\cdot} + OH^-$$

To further investigate whether mitochondrial ubisemiquinones can supply e^- for this reaction we have followed the "oxidant induced reduction" of cyt b. The rationale of this experiment is the observation of Wikström and Berden[17] that cyt b reduction of AA inhibited mitochondria increases as a consequence of ubisemiquinone oxidation.

Figure 6 shows the effect of H_2O_2 on the reduction of mitochondrial cyt b_{566}. Oxygen concentration in the reaction system was adjusted such that after AA addition stirring did not affect the steady state. The extra-reduction of cyt b_{566} after mixing H_2O_2 to the reaction system strongly supports the possibility of H_2O_2 to operate as an oxidant of redox-cycling ubiquinone. To confirm the existence of an electron transfer from semiquinones to H_2O_2 we have confined our reaction system by replacing mitochondria with semiquinones formed in the same reaction system as in Figure 5. (see Figure 7A) Addition of H_2O_2 to this reaction system (Figure 7B) resulted in the more or less complete disappearance of the semiquinone related ESR line while novel spectra of DMPO-spin-adducts occurred which were all indicative of the existence of free OH· radicals. Spectra "a" were obtained by directly trapping OH·

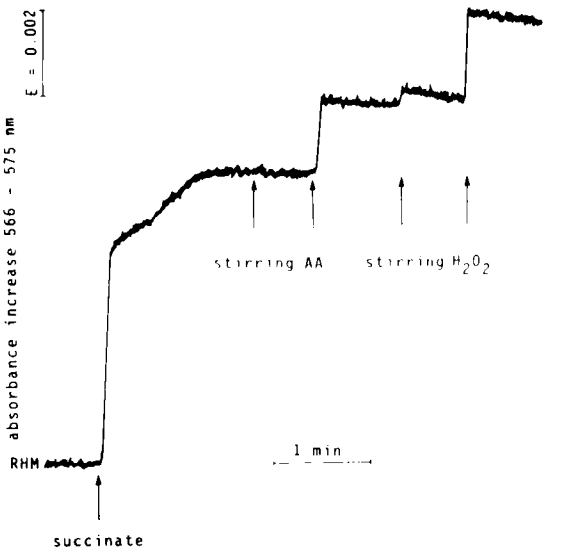

FIGURE 7 Influence of H_2O_2 on the reduction state of cytochrome b_{566}. Experimental details are described in the text and in.[6]

with DMPO while spectra "b" indicated DMPO spin adducts of a hydroxy-ethyl radicals in the presence of ethanol and "c" the formation of carboxyl radicals generated from a reaction of free $OH^{\cdot}$ with added formate.

The formation of this highly toxic oxygen species in the presence of semiquinones and H_2O_2 strongly suggests that electrons of the respiratory chain can be transferred out of the UQ-loop to H_2O_2 as well. In contrast to oxygen data of the literature do not contradict thermodynamically the feasibility of an e^- transfer from semiquinones to H_2O_2. In line with this, there is indirect evidence in the literature that $OH^{\cdot}$ formation from mitochondrial H_2O_2 will also occur in the living cell.[4]

CONCLUSION

- Mitochondrial respiration is associated with a permanent e^--leak out of the UQ-loop under certain conditions.
- Electrons from this loop can be transferred both, to molecular oxygen giving rise to the formation of $O_2^{\cdot-}$ and to H_2O_2 which results in the formation of $OH^{\cdot}$ radicals.
- H_2O_2 which may be produced as a byproduct of respiration can serve as an e^--acceptor of redox-cycling ubisemiquinone.
- A possible role of low potential cyt b as an e^- donor for oxygen out of sequence cannot be excluded but requires further studies.
- Whether or not molecular oxygen will accept electrons from ubisemiquinones seems to depend on the availability of protons at domains where UQ undergo one electron redox cycling.

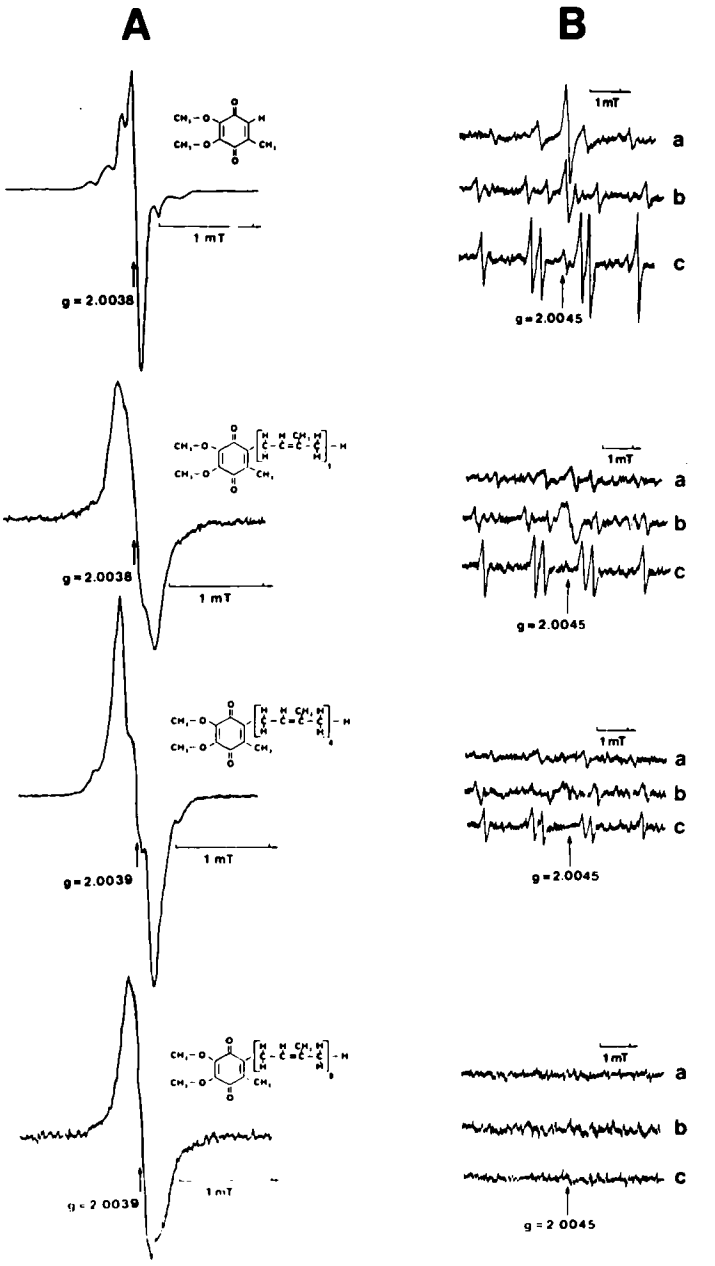

FIGURE 8 ESR-spectra of paramagnetic compounds involved in quinone-catalysed Haber-Weiss reactions in the presence of $OH^{\cdot}$ radical scavengers. Experimental details are described in the text and in.[21]

Acknowledgements

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