Free Rad. Res. Comms., Vol. 8, No. 4-6, pp. 201-217
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QUINONES, SEMIQUINONE FREE RADICALS AND ONE-ELECTRON TRANSFER REACTIONS: A WALK IN THE LITERATURE FROM PERU TO S.O.D.

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(Received September 27, 1989; accepted October 17, 1989)

QUINONE (CHINON): SOME ETYMOLOGICAL NOTES

1638: The Countess of Chinchon, the Wife of the Spanish Governer of Peru is cured of an attack of fever by the administration of an extract of the bark (the 'kina') of a tree called by the indigenous Quichua indians 'kina-kina', bark of barks. The medicine is said to have been recommended to her by a corregidor (a chief magistrate) who eight years earlier had himself experienced its particular effectiveness.

1640: the Countess brings a supply of the bark back to Europe where it becomes known as Peruvian or Jesuit's bark. A Jesuit missionary had possibly been the first to personally experience the bark's potency as a febrifuge and tonic. Jesuit priests also widely extol the bark's qualities. The tree becomes known commonly as the 'fever tree'. In Spanish 'kina-kina' becomes 'quina-quina'. In England by 1707, the variant spelling 'china-china' is also being used.

1742: Linnaeus in Sweden calls the genus in which the tree is classified, Cinchona. (It is said that this was in honour of the Countess and that surprisingly neither 'cinchona' nor its variant 'chinchona' are etymologically connected to 'quina'.)

1803: an acid, kinic acid, has been isolated as a crystalline salt from a Peruvian bark extract.

1810: Gomez of Lisbon obtains a mixture of alkaloids by treating an alcoholic extract of Peruvian bark with water and then adding a solution of caustic potash: he calls the mixture 'cinchonino'.

1820: Pelletier and Caventou in France show that cinchonino contains two alkaloids which they name 'quinine' and 'cinchonine'.

1838: Woskresensky obtains a yellow crystalline compound on oxidation of kinic acid and calls it 'chinoyl'. Beerzelius later substitutes 'chinon' which is still the name in German. In England the name 'kinone' is adopted and is still being used in 1857.

1862: 'kinic' has become to be replaced by 'quinic' and 'kinone' by quinone. (Figure 1.)

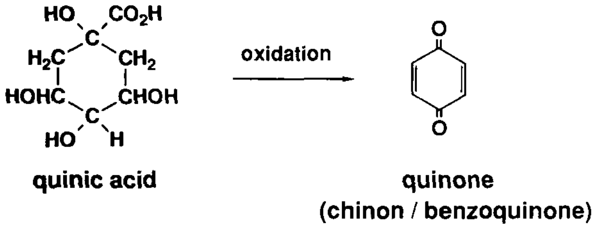

FIGURE 1 Oxidation of quinic acid to quinone.

QUINONES, HYDROQUINONES, QUINHYDRONE AND MERIQUINONES: EARLY OXIDATION-REDUCTION STUDIES

During the second half of the nineteenth century and well into the next a wide variety of substituted benzo-, naphtho-, anthra- and phenanthra-quinones were synthesised. Many were investigated for possible use as dyes in the textile and printing industries. Quinones in general could be readily reduced to hydroquinones. These hydroquinones could be readily reoxidised and the possibility of using them as developers in the rapidly expanding field of photography was soon appreciated (Figure 2).

Many electrochemical studies of quinones were undertaken. Although the term 'quinone', in its strictest sense, continued to refer to compounds containing the doubly unsaturated cyclic carbon ring with two oxygen atoms joined to it by double bonds, it was increasingly clear that many nitrogen-containing compounds without any oxygen atoms also readily underwent reversible reduction and oxidation.[1–4] Such compounds were consequently termed 'quinonoid' or 'quinoid' (Figure 3).

As with the quinones, it was generally accepted that the overall reduction of quinoid compounds involved two electrons. Also as with the quinones, it was clear that their reduction was not a direct single process but involved the formation of an intermediate product. In some instances similar intermediate products were also obtained from mixing the oxidised and reduced forms. In 1908 Willstätter and Piccard

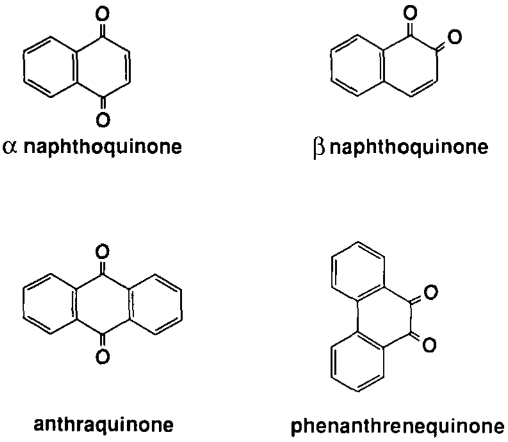

FIGURE 2 Structures of α- and β-naphthoquinone, anthraquinone, and phenanthrenequinone.

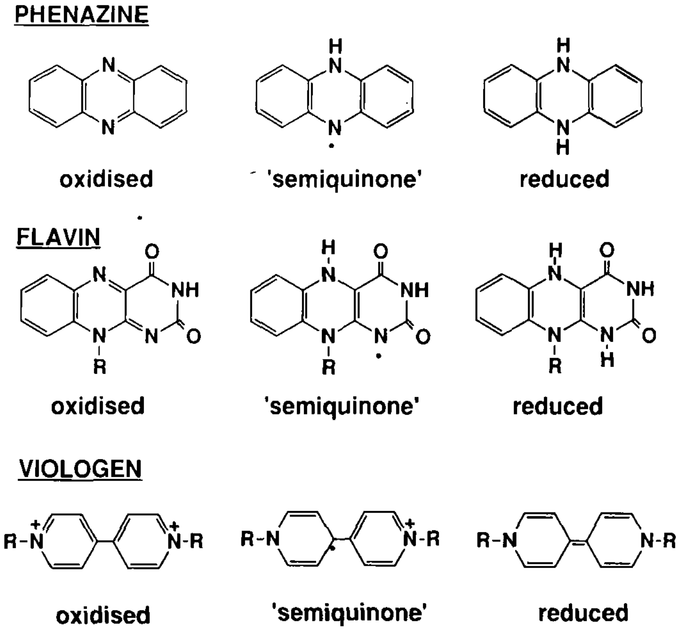

FIGURE 3 Structures of some quinoid compounds and their associated 'semiquinones'.

suggested that the intermediates were made up of two, possibly more, molecules in different oxidation-reduction states. The overall oxidation state was considered to be evenly distributed over the component parts and the general term 'merichinoid' (meriquinone, meros = part) was coined to describe their overall structure.[2]

In the case of benzoquinone, the structure proposed for the intermediate, quinhydrone, was to be the subject of much debate. Could not an intermediate thought to be made up from two molecules in different redox states, be simply an intermediate derived from one molecule in an intermediate redox state – a free radical? (Figure 4).

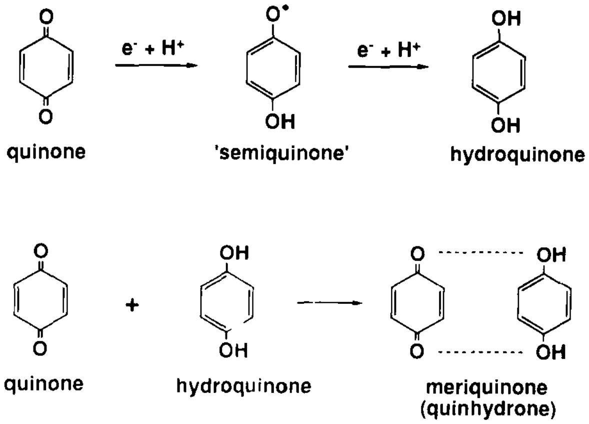

FIGURE 4 Reduction of benzoquinone and the structure of quinhydrone and benzsemiquinone.

THE BIRTH OF 'SEMIQUINONE'

It was in this light that the word 'semiquinone' was born. In 1931 it was first used by Michaelis not to describe an intermediate derived from a quinone, but to one derived from a phenazine compound, pyocanine, a pigment present in the microorganism *Bacillus pyocyaneus.*[5]

During potentiometric studies at the Rockefeller Institute, Michaelis had found that the dyestuff in alkaline solution could be reversibly reduced in the same way as any organic dye of the quinoid type. In acid solution, however, the reduction occurred in two distinct steps each characterised by an individual colour and an individual potential.[6]

alkali	oxidised pyocyanine (blue)		⟶		reduced pyocyanine (colourless)
acid	oxidised pyocyanine (red)	⟶	intermediate pyocyanine (green)	⟶	reduced pyocyanine (colourless)

From a mathematical analysis of the potentiometric titration curves Michaelis concluded that the intermediate compound in acid solution was not a meriquinone made up of the oxidised and reduced forms, but a compound of the same molecular size in 'a true intermediary state of oxidation' – 'a semiquinone'. (Figure 5).

In a stimulating review in 1935 Michelis outlined the various quinoid systems in which semiquinones had been 'identified' as intermediates during reversible oxidation-reduction: phenylene diamines, phenazines, flavins, viologens.[7] In no system could he find any evidence for meriquinone, only for semiquinone, formation. The fact that there was so far no evidence for semiquinones from benzoquinone or other true quinone systems was attributed to the relative instability of many quinones in alkaline solution. Michaelis argued, that such real semiquinones were likely to be anionic. In the same way as the existence of the cationic semiquinones, such as that from pyocyanine, were favoured in acid conditions, the existence of real semiquinones would be favoured in alkalia.

Some quinones did, however, turn out to be sufficiently stable for study and in the next few years experiments with the sulphonates of anthraquinone, naphthoquinone and phenanthrenequinone and with duroquinone (tetramethyl benzoquinone) were to become the focus of much attention (Figure 6).

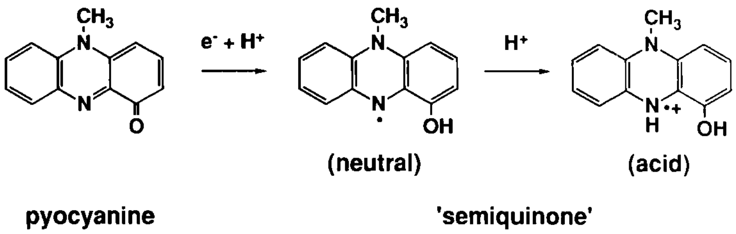

FIGURE 5 Reduction of pyocyanine.

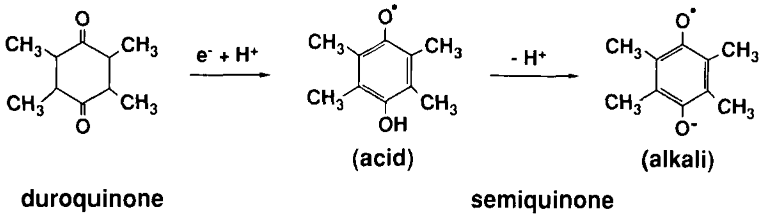

FIGURE 6 Reduction of duroquinone.

'IDENTIFICATION' OF A 'REAL' SEMIQUINONE

The first 'identification' of a 'real' semiquinone was reported in 1936. In a short abstract Hill and Schaffer[8] described how they had noted that of all the oxidation-reduction systems studied potentiometrically, only in the indigo sulphonate system had two separate single valence steps been seen in alkaline solution. In an attempt to find other systems which behaved similarly they had resurveyed the electrochemical behaviour of the anthraquinone sulfonates previously investigated by Conant and colleagues.[3,4] They had concluded that the changing slopes of the titration curves with pH, previously observed, were due to semiquinone formation and not meriquinone formation, as had been previously thought.

Later in 1936 Michaelis, acknowledging the work of Hill and Schaeffer, described how he too had reinvestigated in more detail some earlier electrochemical work, this time with β-naphthoquinone sulphonate.[9] When the yellow dyestuff was reduced in acid solution an ordinary two-electron reduction to the almost colorless leuko dye took place. However at pH = 9–12, an intermediate intense brown color arose which faded on complete reduction. From the titration curves he had also concluded that a semiquinone was formed in alkaline solution.

But were such transient colours solely due to semiquinone radicals and their quinone and hydroquinone companions? Was not there just some possibility that 'valence-saturated dimers' of the merquinone or similar type could also be formed but for some reason were not detectable in such potentiometric studies. After all, no radicals had been directly identified as such. They had only been postulated to explain a still unproven analytical model.

Subsequent papers published in 1937 on phenanthrenequinone-3-sulfonate showed that Michaelis himself was now beginning to have doubts concerning the exclusivity of semiquinones as intermediates.[10] Remembering that other well documented free radicals such as triphenylmethyl had long been known to exist in equilibrium with their dimeric valence-saturated compounds, the Rockefeller group began examining the possibility that semiquinones could behave similarly. It was argued that since dimerization was a bimolecular reaction, the extent to which it occurred would depend on the radical concentration. Hitherto, because of the limited solubility of many quinones, all studies had been made in rather dilute solutions (up to 3 mM). With phenanthrenequinone sulfonate the range of investigation could be considerably extended.

Solutions of the dye were first reduced with hydrogen. The hydroquinone formed was titrated with potassium ferricyanide and the shapes of the potentiometric curves analysed. In acid solution with high initial concentrations it was concluded that significant concentrations of dimer and only an exceedingly small concentration of semiquinone could be formed. In alkaline solution, with hydroquinone concentrations less than 1 mM, only the semiquinone was present in appreciable amounts but above 1 mM approximately equal concentrations of the dimer could also be formed.

In now accepting that dimers as well as semiquinones could exist, Michaelis and Fletcher proposed 'an extension and generalisation of the semiquinone theory'. Seemingly knowing that others would see very little difference structurally, between dimers and the meriquinones of Willstätter and Piccard, they added the comment' 'this theory is by no means a return to the previously accepted assumption that the intermediate forms of oxidation reduction are essentially bimolecular compounds'. Later they went on: 'It must be emphasize tht this theory has little in common with the old quinhydrone theory which neglected the radical form, in as much as we postulate a mobile equilibrium between the two intermediate forms'.

MAGNETIC SUSCEPTIBILITY STUDIES: U-TUBES AND THE GOUY BALANCE

Today the sophisticated study of free radicals in both solids and liquids by electron spin resonance spectroscopy is common place. In 1938 things were not anything like so simple.

It had been appreciated for some time that a free radical, because of its unpaired electron, possesses an overall permanent magnetic moment and as such will behave like a small bar magnet. It is said to be paramagnetic and if placed in a uniform magnetic field it will tend to orient itself in the direction of the field. If placed in a field of varying intensity it will tend to move from the weaker field to the stronger. In contrast, a normal molecule not carrying a permanent magnetic moment when placed in a magnetic field is repelled. The orbiting electrons take up new orbits, with the result that the intensity of magnetisation is less than that of the applied field: it is said to be diamagnetic.

In 1938 the methods in use for measuring the degree to which substances were diagmagnetic or paramagnetic (their magnetic susceptibility) were still relatively simple. Obtaining accurate results was not easy. The first method developed by Gouy in 1889 was principally suited for solids and relied on suspending a rod or glass tube of the substance from one arm of a sensitive balance in such a way that its tip hung between the poles of a powerful electromagnet. On switching on the magnet, the changes in the weight required to counterbalance the rod was a measure of the susceptibility.

Michaelis and collegues anticipated that the diamagnetic susceptibility of a dimeric valence-saturated compound would be an order of magnitude lower than the paramagnetic susceptibility of a radical. Because of the comparatively low free radical concentration this paramagnetism would still be greatly outweighed by the diamagnetic suseptibility of the solvent. The formation of a radical would therefore only be detected as very small changes in the diamagnetic susceptibility of the whole solution. Any traces of paramagnetic impurities might also be troublesome.

To overcome these difficulties it was decided not to use the Gouy method initially but to use a related method, a modification of the U-tube manometric balance method developed by Wills and Boeker.[11] The reduction of the quinone would be undertaken in situ over several hours. Over the whole period no overall change in the susceptibility would be expected. However, if a semiquinone free radical should be formed as an intermediate, then during the first half of the reduction the overall diamagnetic suseptibility should decrease. As the radical is further reduced the diamagnetic susceptibility should increase. In short, a plot of the change in susceptibility with time would go though a maximum and return to base.

And this is just what was observed when the phenanthrene quinone-3-sulphonate was reduced by glucose in alkaline solution. At the same time the original yellow colour changed to an intense black brown and then faded again to a light yellow. Significantly, as the initial quinone concentration increased, the maximum changes in susceptibility observed relative to the initial quinone concentration became smaller, in agreement with the simultaneous presence of increasing amounts of dimer.[12]

On repeating the studies in acid solution using methylglyoxal as a reductant no evidence was found for any significant radical formation. A transient brown colour again appeared and disappeared, this time presumably entirely due to the dimer.[13]

Later in 1938 further studies with duroquinone were reported which may well have been prompted by a paper published earlier in the year by James and Weissberger. These authors had indicated how substitution of the ring hydrogens of benzoquinone by methyl groups increase its stability in alkaline solution.[14] With duroquinone no evidence was obtained for dimer only semiquinone formation. This and the fact that even in acid solutions no crystalline quinhydrone compound could be precipitated on mixing solutions of duroquinone and its hydroquinone, was attributed to steric hinderance from the 'voluminous side chains'.[15]

Interestingly in the same paper Michaelis and colleagues make it clear that they accept that quinhydrone from benzoquinone is a dimeric compound in which the quinone and hydroquinone are linked by two hydrogen bonds. In a footnote they point out that a Dr Coryell in Professor Pauling's laboratory had, at their request, measured the susceptibility of crystalline benzquinhydrone and found it to be diamagnetic. De facto, they now recognised James and Weissberger's 1908 meriquinone structure.

A degree of consensus had at last emerged. During the next ten years the Rockefeller group was to continue these quinone studies using the Gouy balance as well as the U-tube method.[16,17] Such studies however, were soon to be overtaken by the advent of electron paramagnetic (electron spin) resonance spectroscopy.

ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY

"Semiquinones formed by the oxidation of hydroquinones or the reduction of quinones in alkaline media have been studied by paramagnetic resonance absorption spectroscopy. The *p*-benzosemiquinone ion has been observed for the first time in solution; its existence has been established by examination of the structure of its paramagnetic resonance spectrum".

So began the paper by Venkataraman and Fraenkel[18] published in 1947 – another milestone in quinone free radical chemistry. Solutions of hydroquinone in water or ethanol had been treated with a few drops of sodium hydroxide in the presence of air. A yellow colour had developed immediately and after two minutes, the time taken to position the sample and adjust the spectrometer, a strong paramagnetic absorption had been osberved. In some instances the intensity had corresponded to a radical concentration as high as 5 mM. Interestingly in the published paper the absorption

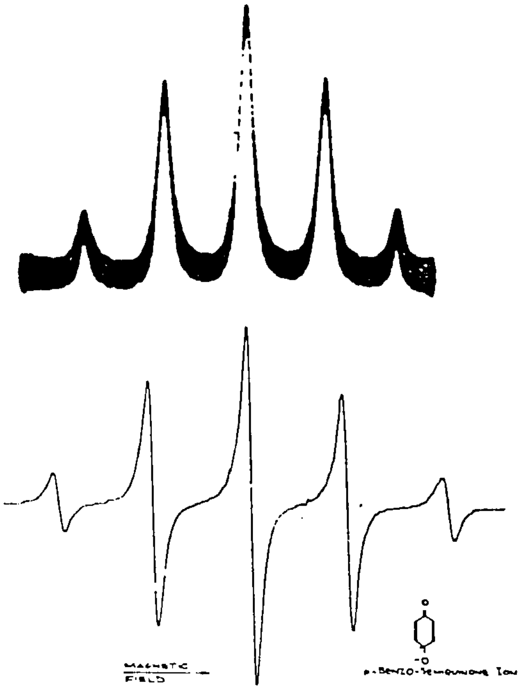

FIGURE 7 The epr spectrum of benzsemiquinone of Venkataraman and Fraenkel.[18]

spectrum is published both in the standard derivative form common today, as well as in the original non-derivatised form. The five lines are very clear. The position of the central peak, the relative heights of the peaks and the space between them are in good agreement with what would be expected from theory (Figure 7).

Related spectra were also obtained for the semiquinones of duroquinone and phenathraquinone using alkaline glucose as the reductant. The intensity of the spectra rose and fell in full agreement with the earlier experiments of Michaelis and colleagues.

So, the presnce of semiquinone free radicals in solution had at last been demonstrated unequivocally, at least at high pH.

OPTICAL ABSORPTION STUDIES OF SEMIQUINONES

Electron paramagnetic resonance spectroscopy was subsequently to prove extremely valuable in the structural characterisation of semiquinone free radicals and in their identification in various biochemical systems. It was to be absorption spectroscopy, however, that was eventually to provide the vast majority of the kinetic and thermodynamic information.

What seems to have been the first absorption spectrum of a true semiquinone was published by Baxendale and Hardy in 1953. Advantage was again taken of the relative stability of duroquinone and durohydroquinone in alkaline solution as well as of the previous proposal of Weiss later supported by the studies of James and Weissberger and Michaelis and colleagues, that in alkaline solution these species will be in equilibrium with the semiquinone'.[15,16,19]

$$QH_2 \rightleftharpoons QH^- + H^+$$

$$QH^- \rightleftharpoons Q^{2-} + H^+$$

$$Q + Q^{2-} \rightleftharpoons 2Q^{\cdot -}$$

Successive amounts of a solution of the hydroquinone were added anaerobically to a fresh solution of the quinone in caustic soda. An intense yellow colour developed (λ max 440 nm) which was attributed to the ionised durosemiquinone ($DQ^{\cdot -}$).

The determination of absorption spectra in neutral and acid solution, however, had to await the coming of the fast reaction technique of flash photolysis.

FLASH PHOTOLYSIS AND THE EFFECTS OF LIGHT

In retrospect it is not surprising that quinones were among the first compounds investigated by this powerful technique. It has been known since the nineteenth century that, in the presence of light, many dyes hastened the deterioration of textiles. Model experiments as early as 1901 had shown that quinones can catalyse the light induced oxidation of isopropanol.[22]

In 1954 Bolland Copper showed that in the case of ethanol, photodegradation can proceed through the reaction of an excited quinone molecule with the alcohol.[23] An alcohol radical is formed which can then react with oxygen leading to acetaldehyde. In the absence of oxygen the alcohol radical can react with the quinone to form the semiquinone. Thus by exposing quinone solutions to a short flash of light and at the same time following the optical absorption on a spectrograph, the spectrum of the quinone free radical can be measured even in neutral or acid solution.[24]

In 1958 papers by Porter and Windsor showing the spectra of a variety of semiquinones were to be the forerunners of a large number of flash photolysis papers from many branches of chemistry.[24,25] The technique was to also led to the development of its high energy analogue, pulse radiolysis which in turn would result in a wealth of new information concerning the reaction of quinones in solution.

PULSE RADIOLYSIS STUDIES

In many ways the pulse radiolysis technique has shown itself to be considerably more versatile than flash photolysis. This is simply because of the way the free radicals are formed initially. In the case of flash photolysis the solution must have a convenient absorption band (chromophore) which the light can excite. The presence of these excited states may also complicate the interpretation of the spectra observed.

In the pulse radiolysis of aqueous solutions, free radicals are the only significnat intermediate species formed in appreciable amounts on the ionization of water. The solute itself is only directly affected by the radiation at vary high concentrations and these are rarely used.

A study of the oxidative effects of the continuous radiolysis of hydroquinone solutions was published in 1959.[26] In 1967 Adams and Michael reported the first pulse radiolysis determination of the absorption spectrum of a real semiquinone. A solution of benzoquinone and benzhydroquinone were exposed to a short pulse of radiation. The conditions were such that the solvated electron and the hydroxyl radical formed from the water reacted with the oxidised and reduced species respectively to form the same semiquinone intermediate.[27]

$$H_2O \rightarrow OH^{\cdot} + e^-_{aq} + H^+$$

$$OH^{\cdot} + QH_2 \rightarrow QH_2(OH)^{\cdot}$$

$$QH_2(OH)^{\cdot} + OH^- \rightarrow Q^{\cdot -} + 2H_2O$$

$$e^-_{aq} + Q \rightarrow Q^{\cdot -}$$

Subsequent studies showed that similar spectra could be observed if only the quinone was present together with excess formate, methanol, ethanol, isopropanol, or *t*-butanol as hydroxyl radical scavengers. Except for *t*-butanol which forms a relatively inconsequential free radical in these systems, such scavengers form organic radicals which then react further with the quinone. Sometimes nitrous oxide or acetone were also present as solvated electron scavengers, the former resulting in the production of additional hydroxyl free radicals, the latter isopropanol free radicals.[28-34]

Using these systems the spectral characteristics of a large variety of semiquinones were determined. Measurements over a range of pH enabled many free radical acid dissociation constants to be determined with considerable accuracy[33] Perhaps more importantly in the context of free radical biochemistry, studies on electron transfer reactions in the presence of oxygen were to lead, as we shall see later, to the routine use of a range of systems for the determination of one-electron redox potentials.

BIOLOGICAL GENERATION OF SEMIQUINONE FREE RADICALS

a) Enzyme oxidation of hydroquinones

Semiquinone free radicals were among the first biochemically generated free radicals to be characterised by electron paramagnetic resonance.

In 1960 Nakamura reported that on rapidly mixing hydroquinone with buffer in the presence of air, 1 μM semiquinone was formed by autoxidation in 4 minutes. In the presence of the enzyme laccase, 3 μM was present within 30 seconds. Further studies with a flow system convinced him that laccase was catalysing the formation of the semiquinone which then dismutated to form the hydroquinone and benzoquinone.[35]

Also in 1960 Yamazaki and colleagues reported how, on preparing a solution of benzhydroquinone, hydrogen peroxide and Japanese turnip peroxidase at pH = 7, a five-line spectrum corresponding to benzsemiquinone formed within 30 seconds and remained for over 2 hours.[36] Although a similar signal was observed without the enzyme this took five minutes to form. When the experiment was repeated at pH = 6 negligible signal was observed in the absence of the enzyme. Although the signal was still formed in the presence of the enzyme, it was smaller and disappeared in less than 2 minutes. Studies at pH = 4.8 were also undertaken. Here solutions had to be flowed through the actual spectrophotometer in order to detect the rapidly decaying signal. The following mechanism was proposed:

$$\text{Peroxidase} + H_2O_2 \rightarrow \text{Compound I}$$

$$\text{Compound I} + QH_2 \rightarrow \text{Compound II} + +QH^{\cdot}$$

$$\text{Compound II} + QH^{\cdot} \rightarrow \text{Peroxidase} + QH^{\cdot}$$

$$QH^{\cdot} + QH^{\cdot} \rightarrow Q + QH_2 \text{ (or } HQ^{\cdot} - QH^{\cdot}\text{)}$$

The results were considered to be completely inconsistent with the possibility that the peroxidase was simply catalysing a two-electron oxidation of the hydroquinone; the semiquinone then being formed by the reaction of the product benzoquinone and hydroquinone.

b) Enzymic reduction of quinones

In 1969 Iyanagi and Yamazaki described how semiquinones could also be formed by the one-electron enzymic reduction of several quinones catalysed by the microsomal flavin enzymes cytochrome b_5 reductase and NADPH-cytochome C reductase.[37] In later papers they describe how NADH dehydrogenase, spinach ferredoxin NADPH reductase, xanthine oxidase and NAPH-lipoamide dehydrogenase can also catalyse one-electron reduction to varying extents.[38–41]

REDOX CYCLING, OXYGEN AND ONE-ELECTRON OXIDATION POTENTIALS

Today the reaction of semiquinones with oxygen is of considerable interest to free radical biologists because of possibility that many toxic compounds may act by what has become known as *redox cycling*. The quinone is reduced enzymatically to a semiquinone; this then reacts with oxygen by an electron transfer reaction. The superoxide radical is generated and the quinone is reformed, ready to undertake the process again (Figure 8).

But what governs such reactions? Do all semiquinones react readily with oxygen? Up until 1969, the time of the discovery of superoxide dismutase, there was a general assumption that they did. We now know that this is not the case. Some semiquinones do, others do not.

The involvement of oxygen in quinone chemistry particularly with respect to the

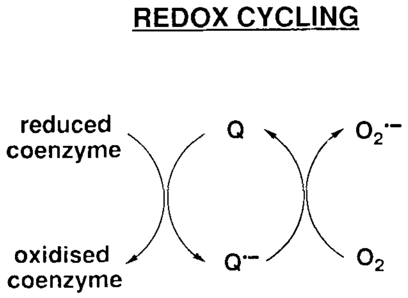

FIGURE 8 Redox cycling of a quinone.

autoxidation of hydroquinones has long attracted considerable attention.[14,42–44] In their paper on the autoxidation of durohydroquinone, James and Weissberger implicated the reaction of the semiquinone with oxygen as a key, but not rate-determining, step in the oxidation: the overall rate is governed by the formation of the semiquinone from the quinone and hydroquinone.[14] In 1947 the actual equation for the transfer of one electron from the semiquinone was written (S-semiquinone, T-solute).

$$S + O_2 \rightarrow T + O_2^{\cdot -}$$

i.e.

$$Q^{\cdot -} + O_2 \rightarrow Q + O_2^{\cdot -}$$

It was stated categorically that the reaction was considered ‘irreversible’.[45]

In 1954 in the photosensitisation paper of Bolland and Copper a similar reaction was proposed.[23] In 1969 Phillips and colleagues published a flash photolysis spectrograph in which an absorption band attributed to a semiquinone can be clearly seen to decay in less than a millisecond in the prsence of oxygen, but over several milliseconds in its absence.

But were such reactions common to all quinones? Pulse radiolysis studies were to show that this was not the case.[32,47] Experiments were being undertaken to find out how some ‘electron affinic’ compounds such as menadione (methyl naphthoquinone) act as radiosensitisers. It has been proposed that such compounds captured solvated electrons and transported them to DNA.[48]

Other studies had indicated, however, that oxygen, the most effective radiosensitiser, reacted with the electron adduct of thymine and thus the proposed mechanisms of sensitisation seemed unlikely.[49] In order to confirm that this reaction was very rapid, pulse radiolysis studies were undertaken to determine the rate constant. Since neither oxygen nor the thymine electron adducts have strong visible absorptions, a competition method was employed using *p*-nitroacetophenone as a reference.[50] This compound was known to react with the thymine electron adduct to form the relatively long-lived and strongly absorbing radical, nitro radical $PNAP^{\cdot -}$. It was anticipated that when increased concentrations of oxygen were present the absorption would be progressively reduced, in agreement with the completing reactions:

$$T^{\cdot -} + RNO_2 \rightarrow T + RNO_2^{\cdot -}$$

$$T^{\cdot -} + O_2 \rightarrow T + O_2^{\cdot -}$$

This was indeed observed and an attempt was made to confirm the result using a different reference compound. Benzoquinone was chosen and a similar competition was again anticipated.

$$T^{\cdot -} + Q \rightarrow T + Q^{\cdot -}$$

$$T^{\cdot -} + O_2 \rightarrow T + O_2^{\cdot -}$$

Surprisingly, however when oxygen was present, even in high concentration, no reduction of the yield of benzosemiquinone radical, $BQ^{\cdot -}$, was observed[32,47] (Figure 9).

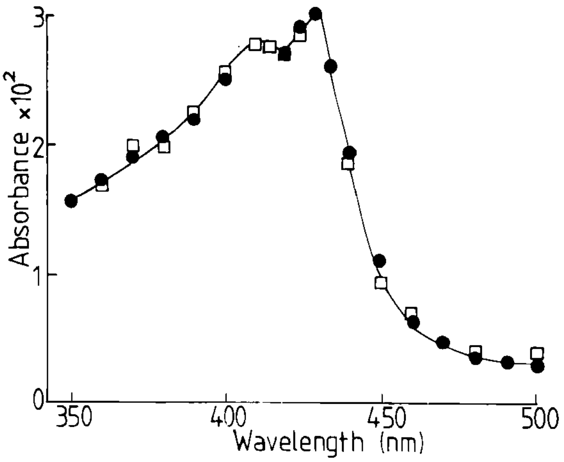

FIGURE 9 The absorption spectrum of benzsemiquinone observed on pulsed radiolysis in the absence and presence of oxygen.

The only explanation was that although oxygen did react rapidly with the adduct, the superoxide free radical produced could go on to react rapidly with the quinone, the exact reverse of what was normally assumed.

$$O_2^{\cdot -} + Q \rightarrow O_2 + Q^{\cdot -}$$

Control studies confirmed the findings and it was clearly of interest to examine other quinones. In the case of anthraquinone sulphonate the reaction did occur as had been indicated previously. With duroquinone, however, an equilibrium situation was observed where the rates of the forward and back reaction were approximately the same.[51]

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

Since the concentrations of all the species present were known, it was possible to determine the equilibrium constant of the equilibrium reaction and hence the relative redox potential $E_0(O_2/O_2^{\cdot -}) - E_0(DQ/DQ^{\cdot -}) = 0.9095\,V$.

Related studies by other workers were to subsequently appear.[52,53] In 1974 Wood, commenting on the accuracy of published values for $E_0(O_2/O_2^{\cdot -})$, calculated, using literature values for the acid dissociation constants of durohydroquinone and for the equilibrium constant of the durosemiquinone dismutation reaction, $E_0(DQ/DQ^{\cdot -}) = 0.25\,V$. Using the above pulse radiolysis data, a value $E_0(O_2/O_2^{\cdot -}) = -0.33\,V$ (oxygen 1 atmosphere, pH = 7) was thus obtained.[54,55]

Since 1974 pulse radiolysis has become the method of choice for determining the one-electron oxidation reduction potentials of a great variety of biological and foreign compounds.[56,57] Such data along with the mass of information emerging from biochemical, toxicological and radiobiological studies since the characteristion of superoxide dismutase,[58] is assisting considerably our understanding of free radical biology in general.

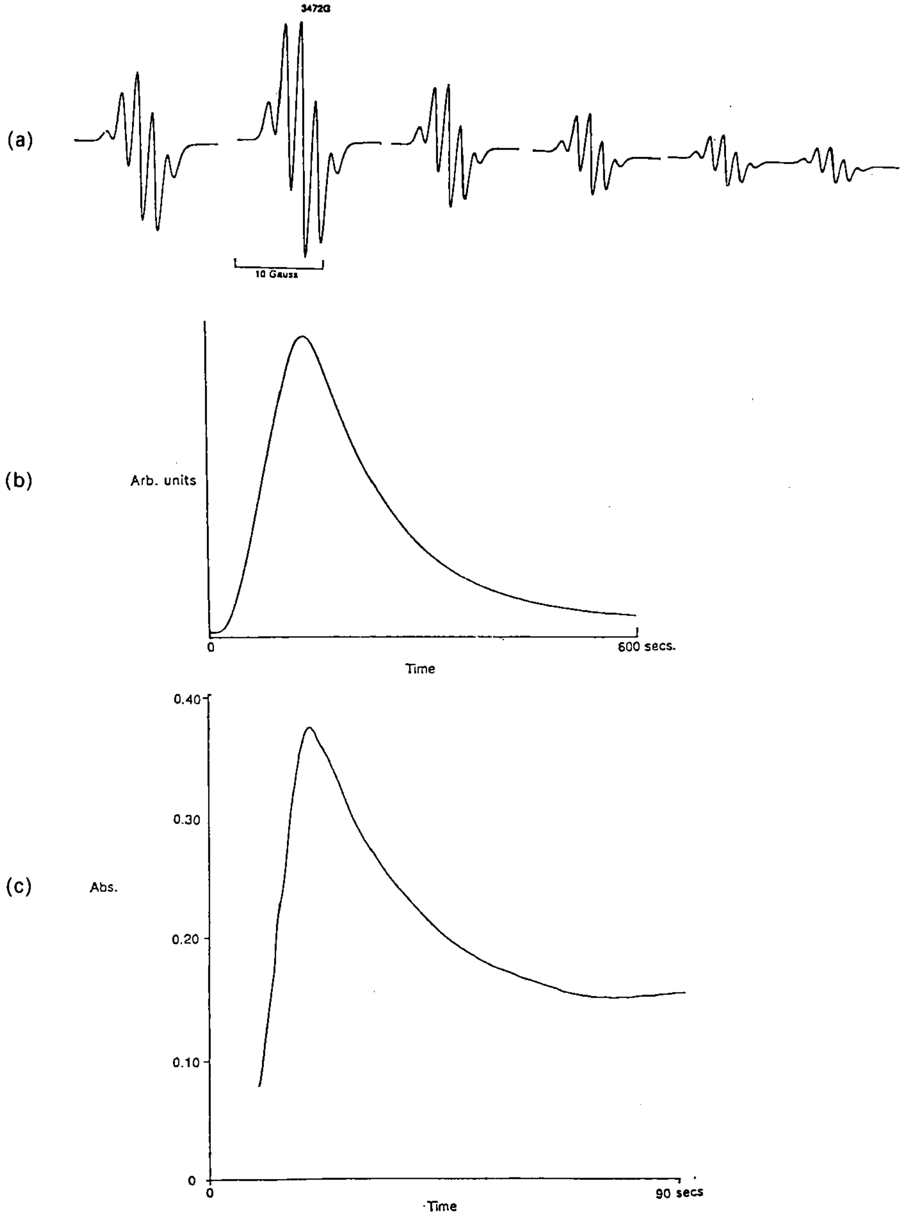

FIGURE 10 Changes in electron paramagnetic (spin) resonance spectra (frequency 9.77 GHz, power 10 dB, modulation 1.0) and optical absorption at 450 nm on reduction of alkaline aqueous ethanol phenanthrenequinone solutions by glucose. a) e.p.r. spectra taken at approximately 1 min intervals; b) change in absorption at 3472.0 gauss (20 s time constant); c) change in optical absorption at 450 nm. For the optical studies, concentrations were as described in the text; for e.p.r. studies, concentrations were adjusted to slow down the reaction.

CONCLUSION

Whilst quinones do have many reactions in common, the particular reactions which will ultimately predominate in any biochemical system will clearly very much depend on the structure of the compound in question. We have come along way from Peru. For those interested in following the trail further the other contributions to this volume and the references cited will, I am sure, make stimulating reading. For those interested in teaching, a variation of the experiment of Michaelis with phenathrenequinone makes an excellent demonstration.[18,59,60] Two solutions are prepared:

A – 25 mgs phenathrenequinone (Aldrich) in 100 ml. ethanol in a 500 ml bottle.
B – 80 ml. 1 N NaOH plus 100 ml. water

A few minutes before needed, solution B is added to A followed by 4 g glucose. The mixed solution is allowed to stand until it goes pale yellow. To demonstrate, the solution is swirled slowly until it turns a dark orange. The reduced quinone, the hydroquinone, is oxidised by the air and an appreciable concentration of dimer and semiquinone develops. When the rate of swirling is increased, the solution goes pale yellow again as the hydroquinone, dimer and semiquinone are completely oxidised to the quinone. On being left to stand, the quinone is reduced by the glucose, the orange colour redevelops and then fades, the solution returning to the original pale yellow of the hydroquinone.

The reduction of the quinone by the glucose following oxygenation, can be readily followed spectrophotometrically at 450 nm or by electron paramagnetic resonance using various quinone, alkali, glucose and oxygen concentrations. Some typical results are shown (Figure 10).

Acknowledgements

I am greatly indebted to the Cancer Research Campaign who supported by work on quinones whilst working at the Gray Laboratory from 1967–1973 and who have since continued to support free radical research at Brunel University. I am also very grateful to the many colleagues shown in the references who have been associated with these studies, to Dr W. Flitter and Mrs K. Kalsi for assistance in obtaining the e.p.r. spectra and to the University library without whom this walk in the literature would never have materialised.

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Accepted by Prof. H. Sies