Semiquinone Anion Radicals of Catechol(amine)s, Catechol Estrogens, and Their Metal Ion Complexes

by B. Kalyanaraman,* C. C. Felix,* and R. C. Sealy*

Introduction

Catechols are widely distributed in nature, in both plant and animal systems. Catechol itself is an environmental cocarcinogen present in tobacco smoke (1-3). In mammals, catecholamines act as neurotransmitters. They find use in medicine as antiparkinsonism drugs (dopa), antihypertensive agents (α-methyldopa), and as bronchodilators (isoproterenol). Antitumor activity of a number of catechols (γ-glutamyl-3,4-dihydroxybenzene, 4-methoxy catechol, etc.) and catecholamines (i.e., dopa methyl ester, dopamine, 3,4-dihydroxybenzylamine, and 5-S-cysteinyldopa) in both in vitro and in vivo systems has been reported (4-9). Side effects have been reported for all these drugs, and include hepatic injury (10), cardiotoxicity (11), hemolysis (12) and photosensitivity (13). These side effects and the cytotoxicity of these drugs in general are thought to be related to the production of damaging free radicals and o-quinones (14-20).

Phenols are precursors to catechols (23-25); they are widely used as antioxidants (21,22,26) and also find use as antitumor agents (5). Several phenolic agents also induce skin depigmentation (27,28). These effects have been attributed to metabolic activation of phenols to various catechols in pigmented systems (5,18). Both estradiol and diethylstilbestrol (DES) are phenolic estrogens and environmental carcinogens (29,30). They undergo biotransformation to catechol estrogens in uterus (31) and in brain tissues (32,33). Free radicals from DES-catechol and 2-hydroxy-estradiol have been implicated in their toxicity (34-36).

Several xenobiotics (benzene, 4-bromobenzene, and acetaminophen) undergo aromatic hydroxylation by cytochrome P-450 in the liver to form the corresponding catechol(s) (37-40). In addition the hematopoietic toxicity of benzene and the nephrotoxicity of bromobenzene has been linked to semiquinone/quinone production from catechol metabolites (37,38).

This review addresses the generation, identification, and reactions of o-semiquinones and o-quinones from catechol(amine)s and catechol estrogens in a biochemical milieu.

Biochemical Toxicology of Semiquinones and Quinones

Catechol(amine)s and catechol estrogens are degraded to semiquinone radicals by both enzymatic and nonenzymatic pathways (Fig. 1). Semiquinone radicals can be formed either directly or indirectly from oxidation of the parent catechol(s) or from reduction of quinones (41-46).

Since catechols induce toxicity in several target organs, they are also likely to undergo metabolic activation in several enzyme systems. Enzyme systems capable of oxidizing catechol(amine)s,catechol estrogens, and phenols in vitro include: cytochrome P-450/NADPH (47-49), horseradish peroxidase (HRP)/H₂O₂ (50), PG-synthase/arachidonic acid (51), catalase/H₂O₂ (52), and tyrosinase/O₂ (41). Those that reduce the quinones are: NADPH-P450-reductase, DT-diaphorase, and xanthine/xanthine oxidase (53-55). One-electron oxidation of phenols leads to aryloxy radicals which through sec-
Secondary reactions can in turn form semiquinones (Fig. 1) (42). In a biological milieu, semiquinones and quinones can bind covalently to proteins, enzymes or other endogenous constituents producing secondary free radicals and products (56–60) (Figs. 2 and 3).

The antitumor effects of several catechol(amine)s have been attributed to inhibition of DNA polymerase (in melanocytes) by o-quinones or o-semiquinones (4,5,58). The neurotoxic effects of 6-hydroxy or amino-substituted catechol(amine)s (i.e., 6-hydroxydopa, 6-amino dopamine, etc.) have also been related to production of free radicals (61–63).

Inactivation of enzymes/proteins by o-quinones is presumably due to the nucleophilic addition reactions (of quinones) to sulfhydryl or amino groups present in these macromolecules (16,58). The production of 5-S-cysteinyl dopa in the urine of melanoma patients (64) and the isolation of quinone-alanine adduct(s) in terrestrial humic acid (65) provide indirect evidence for occurrence of these addition reactions. Reactions between o-quinones and proteins provide a basis for formation of “melano-proteins" (66). The toxicity of adrenochrome (a material which leads to myocardial necrosis) has been attributed to formation of the (one-electron) reduced free radical and to the oxy-radicals derived from it (via redox cycling of oxygen) (67–69).

### Generation of o-Semiquinones

Semiquinones are generated from oxidation/reduction of catechols and quinones by a variety of methods, including photooxidation, autoxidation/chemical oxidation and enzymic oxidation (70–80).

### Photooxidation

Photooxidation provides a simple, clean method for generating semiquinone radicals and studying their reactions. The primary photoresponse in dopa, catechol, and a variety of other catecholamines involves a mixture of photoionization and photohomolysis (81). This was established by a quantitative spin trapping procedure that we developed using 5,5-dimethyl-1-pyrroline N-oxide (DMPO), which scavenges both hydrated electrons and hydrogen atoms formed during the photooxidation.

\[
\begin{align*}
&\text{Ascorbic Acid} \\
&\text{Macromolecules} \quad \text{(proteins, polypeptides)} \\
&\text{Covalent Binding}
\end{align*}
\]

![Figure 2. Possible fates of quinones in biological systems.](image)

![Figure 3. Possible oxidation and reduction pathways of semiquinones.](image)

The quantum yield for semiquinone formation is ca. 0.04 at pH 7, increasing to about 0.08 at higher pH where the catechol moiety is ionized.

Sensitized photolysis of catechols and catecholamines has been demonstrated using a variety of dyes. Visible irradiation in the presence of hematoporphyrin, Rose Bengal, methylene blue, and other sensitizers gives intense spectra of corresponding semiquinones (70). In general, photooxidation (using a slow flow) can be used for (1) generating specific primary radicals for characterization purposes without interference from secondary radicals and (2) obtaining kinetic data for
their reactions, e.g., termination rate constants for o-semiquinones.

**Autoxidation/Chemical Oxidation**

Free-radical chain reactions have been proposed during autoxidation of catechol(amines) (82). From the pH dependence of oxygen consumption, the initiating step of an autoxidation reaction appears to involve electron transfer from the mono-anion of the catechol(amine) to molecular oxygen, e.g.,

\[
\text{HO-R} + \text{O}_2 \rightarrow \text{HO-R} + \text{O}_2^2
\]

A more recent study, however, suggests that this electron transfer to oxygen is metal-catalyzed (83). The superoxide radical formed in the initiating reaction also is scavenged by catechol(amine) (84).

\[
\text{HO-R} + \text{O}_2 \rightarrow \text{HO-R} + \text{H}_2\text{O}_2
\]

Radicals can be generated by either static or flow measurements (85). Static oxidation involves addition of the catechol(amine) or catechol estrogen to an oxygen-saturated solution of sodium hydroxide (76,78,79). Semiquinones are observed fairly easily at high pH because of the slow rate of dismutation of radical anions. Autoxidation also allows the monitoring of secondary radical formation, i.e., semiquinones from 6-hydroxy-substituted catechol(amine). Alkaline hydrogen peroxide (a source of superoxide anion) is another effective way of oxidizing catecholamines and, possibly, catechol estrogens (84). Both sodium periodate and silver oxide are often used to oxidize catechols. Periodate is a two-electron oxidizing agent, but silver oxide appears to act as a one-electron oxidant (75,76).

**Enzymatic Oxidation**

o-Semiquinones formed during enzymatic oxidation have previously been detected by ESR using continuous flow methods (85). This procedure entails the use of large volumes of substrate and enzyme. Recently, we have developed a spin-stabilization procedure by which o-semiquinones (formed enzymatically) can be stabilized by chelation through the use of diamagnetic di- or trivalent ions (73).

Although several metal ions (e.g., Al³⁺, Y³⁺, Cd²⁺, Ca²⁺, Mg²⁺, and Zn²⁺) have been employed to stabilize o-semiquinones in aqueous (86) and nonaqueous media (87), we feel that either Mg²⁺ or Zn²⁺ is more likely to be useful in biological systems, and consequently only Zn²⁺- and Mg²⁺-complexed o-semiquinones in aqueous media are discussed in this review.

Except at high pH, o-semiquinone radicals are transient, decaying rapidly via disproportionation to give the catechol and o-quinone.

\[
\begin{array}{c}
\text{HO-R} + \text{O}_2 \rightarrow \text{HO-R} + \text{O}_2^2 \\
\text{2H}^+ + \text{HO-R} \rightarrow \text{HO-R} + \text{H}_2\text{O}_2
\end{array}
\]

For dopa, the measured radical half-life was 2 msec for a steady-state concentration of 2.5 μM, corresponding to a second-order termination rate constant of \(2 \times 10^9 \text{ M}^{-1} \text{sec}^{-1}\) (Fig. 4). However, Zn²⁺-complexed dopa semiquinone radicals are much less transient than the uncomplexed ones (88). Decay remains second-order, but the radical lifetime is now several seconds for a steady-state concentration of ca. \(10^{-6} \text{ M}\). The calculated second-order rate constant is \(1.1 \times 10^4 \text{ M}^{-1} \text{sec}^{-1}\).

**Figure 4.** ESR spectra \((a,c)\) and kinetic profiles \((b,d)\) for uncomplexed and spin-stabilized o-semiquinones produced by the UV photolysis of dopa. Conditions: \((a,b)\), 25 mM dopa, pH 7.0; \((c,d)\), 25 mM dopa plus 0.08 M Zn²⁺ in acetic acid–acetate buffer, pH 5.0.

Chelation (or complexation) is therefore extremely effective in decreasing the rate of radical termination. The uncomplexed o-semiquinone at neutral pH has a rate constant over 10,000-fold greater. Thus, the complexed radical can be detected at rates of radical formation 10,000 times lower than are necessary to detect...
the uncomplexed o-semiquinone. This allows the use of static rather than flow systems (73).

Whereas HRP/H$_2$O$_2$-dependent oxidation of catechol(amine) and catechol estrogens involves o-semiquinones as obligate intermediates (88), the tyrosinase-catalyzed oxidation proceeds via a two-electron oxidation (41) with the formation of o-semiquinones in a secondary reaction.

Whereas the identification of semiquinones from catechols has been fairly straightforward (71,74), there existed several inconsistencies with regard to interpretation of spectra of o-semiquinones from dopa (89) and epinephrine (91). The major reasons for the observed inconsistencies were: the presence of more than one spectral species; the presence of magnetically inequivalent methylene protons in the amino acid side chain; and acid-base equilibria in the radicals.

For example, oxidation of dopa and its analogs can, depending on the conditions employed, give three major types of radical (D1-D3). The primary radical (D1) does not show the expected multiplicities or linewidths because of a combination of the magnetic inequivalence referred to and restricted rotation (46,90). This phenomenon is illustrated in Figure 6. At low temperature only one-half of the spectral lines are clearly visible, whereas when the temperature is increased the "missing lines" that were previously broadened are apparent and complete spectral analysis becomes possible. An additional complexity in the system is provided by the ionization of the amino group (pK$_a$ = 9) which causes a shift in the spectral parameters at high pH (Fig. 7).

Data for uncomplexed and complexed primary o-semiquinones of catechol(amine)s are given in Tables 1 and 2. The magnetic parameters of the complexes (Table 2) are modified from those of the uncomplexed species, with the differences between them being fairly constant (74). For example, for the Zn$^{2+}$-complexed species $a_3^o$ and $a_4^o$ typically decrease by about 0.28 G in going to the complex while $a_5^o$ increases by about the same amount. $a_6^o$ also increases for Zn$^{2+}$. Observation of satellite peaks from magnetic isotopes present in natural abundance [e.g., from $^{67}$Zn (4%, I = 5/2)] verified that complex formation is occurring. Also indicative of com-

**Characterization and Identification of o-Semiquinones**

Production of primary and secondary free radicals from the oxidation of catechols and the dopa and epinephrine classes of catecholamines is shown in Figure 5.
complex formation are the observed changes in $g$ value. $g$ Values are decreased in the complexes, consistent with spin density in a vacant metal orbital.

Although hyperfine couplings to aromatic protons, in particular $a_{2g}^N$, do not differ markedly for the majority of radicals studied, couplings to the methylene protons in a substituent at position 4 vary considerably, suggesting that the rotations of these protons can be quite restricted (Tables 1 and 2). For example, whereas the protons in a freely rotating alkyl substituent (such as the methyl group in 4-methyl o-benzoquinonone) have a hyperfine splitting of about 4.8 $G$, the protons in the $\alpha$-methyl dopa semiquinone radical [where $R = CH_2C(CH_3)(NH_3^+)(CO_2^-)$] have couplings as low as 2.2 $G$ (the mean of splittings from two inequivalent protons). In general, the methylene proton couplings decrease as the bulk of the substituent on the carbon atom bearing the methylene protons increases.

Secondary radicals C2 from catechols and D2 and D3 from dopa analogs have been detected during autoxidation (44,76) and enzyme oxidation (with tyrosinase); they are derived from hydroxycatechols, 6-hydroxydopamine, and 5,6-dihydroxyindole respectively (Table 3). The unusual spectrum of radical D2 again is a consequence of magnetic inequivalence and restricted rotation in a single radical (Table 3). The spectrum of the cyclized radical, D3, is quite distinctive, showing the hyperfine coupling to nitrogen (Table 4).

Oxidation of epinephrine and its analogs in aqueous solution gives transient, broad, poorly resolved spectra under most conditions (91). Use of ESR-spin stabilization, however, enabled us to obtain three types of radical (75). Their spectra and, for the most part, their structures, are distinct from the dopa series.

The primary radical E1 lacks a second methylene hydrogen (Fig. 5) in the side chain, so that its spectrum is much narrower than that of D1; spectra of the secondary radicals (E2 and E3) are, however, much greater because of a large hyperfine splitting from nitrogen following cyclization (note that the nitrogen coupling in D3 is much lower than in either E2 or E3 possibly due to additional spin-delocalization in the indole ring). Also notable is the failure to detect hydroxy-substituted semiquinones from the epinephrine class unlike the dopa or catechol series (Fig. 5). This is possibly due to a more rapid rate of cyclization of the precursor quinones. Magnetic parameters that characterize these secondary radicals are given in Table 5.

Adrenochrome is the stable 4-electron oxidation product of epinephrine. There is evidence for increased toxicity (e.g., myocardial necrosis) from adrenochrome in the presence of the reducing agents, ascorbic acid and cysteine (67). Ascorbic acid previously has been shown to reduce adrenochrome to leucodrenochrome via a free radical mechanism. Adrenochrome forms both one-electron reduced and one-electron oxidized radicals.
Table 1. Electron spin resonance data for primary o-semiquinones (uncomplexed) from catechol, dopa, and epinephrine and their analogs.

| Parent catechol(amine) | R in radical I | α² | α² | α² | α² | α² | α² | α² | α² | α² | α² | g   |
|------------------------|----------------|----|----|----|----|----|----|----|----|----|----|-----|
| Catechol               | H              | 0.76 | 3.66 | 0.76 | 3.66 | 2.0046 |
| 3,4-Dihydroxybenzoic   | CO₂           | 0.72 | 3.16 | 1.22 |     | 2.0047 |
| acid                   |               |     |     |     |     |     |     |     |     |     |     |     |
| 3,4-Dihydroxyhydro-   | CH₃CH₂CO₂⁻    | 0.22 | 3.73 | 0.88 | 3.56 | 2.0045 |
| cinnamic acid          |               |     |     |     |     |     |     |     |     |     |     |     |
| 3,4-Dihydroxybenzyl    | CH₃NH₄⁺       | 0.82 | 3.48 | 0.93 | 2.75 | 1.38[a⁡N] | 2.0045 |
| amine                  | CH₂NH₂        | 0.34 | 3.72 | 0.79 | 4.13 | 0.79[a⁡N] | 2.0045 |
| 3,4-Dihydroxyphenyl-   | CH₃CO₂⁻       | 0.29 | 3.71 | 0.89 | 3.12 | 1.15(a⁡OCH₃) | 2.0045 |
| alactic acid           |               |     |     |     |     |     |     |     |     |     |     |     |
| 4-Methoxycatechol      | OCH₃          | 0.61 | 4.19 | 1.30 |     |     |     |     |     |     |     | 2.0045 |
| Dopa                   | CH₃CH(NH₄⁺)CO₂⁻ | 0.51 | 3.58 | 0.94 | 1.94,3.34 |     |     |     |     |     |     |     |
|                        | CH₃CH(NH₃⁺)CO₂⁻ | 0.35 | 3.71 | 0.88 | 2.32,3.15 |     |     |     |     |     |     |     |
| α-Methyldopa           | CH₃C(CH₃)₂NH₄⁺CO₂⁻ | 0.59 | 3.55 | 0.94 | 1.70,2.70 | 2.0045 |
|                        | CH₃C(CH₃)₂NH₃⁺CO₂⁻ | 0.42 | 3.69 | 0.89 | 2.00,2.82 | 2.0045 |
| Dopa methyl ester      | CH₃CH(NH₃⁺)CO₂CH₃ | 0.42 | 3.65 | 0.93 | 2.54,2.66 | 2.0045 |
| Dopamine               | CH₃CH₂NH₃⁺     | 0.46 | 3.58 | 0.94 | 2.98 | 2.0045 |
|                        | CH₃CH₂NH₂     | 0.27 | 3.73 | 0.89 | 3.13 | 2.0045 |
| 3,4-Dihydroxymandelic | CH(OH)CO₂⁻   | 0.55 | 3.53 | 0.86 | 1.89 | 2.0045 |
| acid                   |               |     |     |     |     |     |     |     |     |     |     |     |
| 3,4-Dihydroxyphenyl-   | CH(OH)CH(NH₃⁺)CO₂⁻ | 0.48 | 3.71 | 0.85 | 3.12 | 2.0045 |
| serine                 |               |     |     |     |     |     |     |     |     |     |     |     |
| 6-Hydroxydopamine      | CH₂CH₂NH₃⁻,5-O⁻ | 0.58 | —   | 0.87 | 3.24 | 2.0043 |
| 6-Aminodopamine        | CH₃CH₂NH₃₂⁻,5-NH₂ | 0.77 | —   | 1.60 | 2.75 | 2.75[a⁡N], 1.34[a⁡NH] | 2.0044 |
| Epinephrine            | CH(OH)CH₂NHCH₃ | 0.60 | 3.62 | 0.83 | 2.31 | 0.18,0.26 | 2.0044 |
|                        | CH(O⁻)CH₂NHCH₃ | 0.56 | 3.58 | 0.86 | 2.26 |     | 2.0044 |
| Norepinephrine         | CH(OH)CH₂NH₂ | 0.60 | 3.62 | 0.82 | 2.30 | 0.12,0.26 | 0.09[a⁡N] | 2.0044 |
|                        | CH(O⁻)'CH₂NH₂ | 0.46 | 3.58 | 0.84 | 2.22 |     | 2.0044 |
| Isoproterenol          | CH(OH)CH₂NHCH₂CH₃ | 0.60 | 3.60 | 0.80 | 2.28 | 0.20,0.20 | 2.0044 |
|                        | CH(O⁻)'CH₂NHCH₂CH₃ | 0.48 | 3.53 | 0.82 | 2.20 | 0.18,0.26 | 2.0044 |
| 3,4-Dihydroxynor-      | CH(OH)CH(CH₃)NH₂ | 0.56 | 3.61 | 0.85 | 1.96 | 2.0045 |
| ephedrine              |               |     |     |     |     |     |     |     |     |     |     |     |

The one-electron oxidized species has recently been identified during peroxidatic oxidation of adrenochrome (75). However, there is evidence for one-electron reduction of adrenochrome in microsomes containing NADPH (68). This proposed species apparently is oxidized by molecular oxygen forming superoxide and the parent compound (68). We have again chosen the spin stabilization approach in a chemical system (92) (Fig. 8). Magnetic parameters for the Zn²⁺- and Mg²⁺-complexed species are in Table 6.

Semiquinone radicals have been detected for the first time from auto- and enzymatic oxidation of catechol estrogens (93); the spin stabilization approach was again crucial to detect semiquinones produced enzymatically. Using either Zn²⁺ or Mg²⁺ as complexing agents, we showed the production of semiquinones (VII (R = H), VIIa (R = H), VII (R = OH) and VIIa (R = OH)) during peroxidase/tyrosinase oxidation of catechol estrogens and estrogens (Figs. 9 and 10). The species VII (R = H) is characterized by three large hyperfine couplings to β-alicyclic protons (at C-6 and C-9), whereas VII (R = OH) exhibits only two large couplings. The species VIIa (R = H) is characterized by only one large coupling to an alicyclic β-proton (C-9) and a significant coupling to an aromatic proton; species VIIa (R = OH) exhibits a similar pattern. From these spectra, one can unequivocally assign the coupling to specific alicyclic protons. The g-values are close to those reported for metal complexes of o-semiquinones from simple catechols (Fig. 10) (71,74). Magnetic parameters that characterize each species are given in Table 7.

Reactions of α-Semiquinones

The spin stabilization approach has enabled us to study radical reactions of α-semiquinones in two enzymatic systems (HRP/H₂O₂ and tyrosinase/O₂). α-Semiquinone radicals were detected in high steady-state con-
Table 2. Electron spin resonance data for Zn$^{2+}$-complexed primary o-semiquinones from catechol, dopa, and epinephrine and their analogs.

| Parent catechol(amine) | R in radical II | $a^g$ | $a^h$ | $a^s$ | $a^h$ | $a_{other}$ | $g$  |
|------------------------|-----------------|-------|-------|-------|-------|-------------|-----|
| Catechol               | H               | 0.5   | 3.9   | 0.5   | 1.35  | (a$^{OCH_3}$) | 2.0040 |
| 4-Methoxycatechol      | OCH$_3$         | 0.75  | 4.51  | 0.97  | 1.28  | (a$^{N}$N) | 2.0040 |
| 3,4-Dihydroxybenzoic acid | CO$_2$         | 0.47  | 3.65  | 0.85  | 1.28  | (a$^{N}$N) | 2.0040 |
| 3,4-Dihydroxyhydrocinamic acid | CH$_2$CH$_2$CO$_2$ | -     | 4.02  | 0.62  | 4.02  | 2.0038 |
| 3,4-Dihydroxybenzylamine | CH$_2$NH$_2$ | 0.52  | 3.65  | 0.71  | 3.05  | 0.23 | 2.0039 |
| 4-Methylecatechol      | CH$_3$          | <0.2  | <0.2  | <0.2  | <0.2  | <0.2 | 2.0040 |
| Dopa                   | CH$_2$CH(NH$_2$$_2$)CO$_2$ | 0.25  | 3.83  | 0.67  | 2.65, 3.11 | 2.0039 |
| Dopa methyl ester      | CH$_2$CH(NH$_2$$_2$)CO$_2$ | <0.2  | 3.9   | 0.6   | 2.2, 4.2 | 2.0039 |
| Dopamine               | CH$_2$CH$_2$NH$_3$ | 0.31  | 3.82  | 0.66  | 1.84, 2.96 | 2.0039 |
| 6-Hydroxydopamine      | CH$_2$CH$_2$NH$_3$, 5'-O' | 0.31  | 3.76  | 0.71  | 2.63, 2.95 | 2.0039 |
| Epinephrine            | CH$_2$CH$_2$NH$_3$ | 0.16  | 3.88  | 0.67  | 3.36  | 0.22 | 2.0039 |
| Norepinephrine         | CH$_2$CH$_2$NH$_3$ | 0.22  | 3.62  | 0.65  | 3.15  | 0.23 | 2.0039 |
| Isoprotorenol          | CH$_2$CH$_2$NH$_3$ | 0.38  | 3.70  | 0.65  | 3.10  | 0.26 | 2.0039 |
| Dihydroxy nor-ephrine  | (CH$_2$)$_2$NH$_3$ | 0.38  | 3.70  | 0.65  | 3.10  | 0.26 | 2.0039 |
| Adrenalone             | CH$_2$CH$_2$NH$_3$ | 0.36  | 3.70  | 0.60  | 3.70  | 0.26 | 2.0039 |
|                        | CH$_2$CH$_2$NH$_3$ | 0.55  | 2.90  | 1.65  | -     | 0.22, 0.20 | 2.0041 |

From photolytic systems, the rate constant $k_d$ was calculated as ca. $10^4$ M$^{-1}$ sec$^{-1}$ (for complexed semiquinones) and as $10^5$ M$^{-1}$ sec$^{-1}$ for uncomplexed semiquinone radicals.

During peroxidatic oxidation, with H$_2$O$_2$ limiting and constant substrate concentrations, the duration of the steady-state ($t_{ss}$) is linearly dependent on the initial concentration of hydrogen peroxide, [H$_2$O$_2$]. This allowed an estimate (Fig. 11) of the rate of the rate of removal of hydrogen peroxide in this system, since

$$\frac{-d[H_2O_2]}{dt} = v = \frac{[H_2O_2]_0}{t_{ss}}$$

Correlation of this rate with the measured rate of radical formation (by obtaining the termination rate constant and steady-state radical concentrations) verified that the semiquinone is an obligate intermediate in the reaction.

In systems containing catecholamine, HRP/H$_2$O$_2$, and phenol in moderate concentrations, the rate of semiquinone production showed a marked increase as a function of phenol concentration. This is consistent with the production of phenoxy PhO' radicals which subsequently oxidizes the catecholamine:

$$\text{PhOH} \xrightarrow{-e^{-}} \text{PhO}'$$

$$\text{PhO'} + QH_2 \rightarrow \text{PhOH} + Q' + 2H^+$$

In contrast, ascorbate (Fig. 12) and glutathione resulted in a lag time for semiquinone detection that was proportional to the concentration of reductant added. During the lag time, only radicals from the reducing agent are detected. Reaction between $o$-semiquinone and ascorbate was reported to be too slow to measure by pulse methods, although there is ESR evidence for the reduction of ascorbate by a semiquinone radical from 6-hydroxydopamine (94).

**Figure 8.** Free radical from reduction of adrenochrome.
Table 3. Electron spin resonance data for secondary o-semiquinones from the autoxidation of catechol(amine)s.

![Chemical structure](image)

| Parent catechol(amine)               | R in radical III | Hyperfine couplings, G |
|--------------------------------------|------------------|------------------------|
| 3,4-Dihydroxyhydrocinnamic acid      | CH₂CH₆COO⁻       | a_H² 0.55, a_H⁴ 0.81, a_H¹ 4.22, a_H¹ 0.11, a_III 2.0044 |
| 3,4-Dihydroxycinnamic acid          | CH=CHCOO⁻        | a_H² 0.40, a_H⁴ 1.37, a_H¹ 2.92, a_III 1.80, a_III 2.0044 |
| 4-Methylcatechol                     | CH₃              | a_H² 0.58, a_H⁴ 0.65, a_H¹ 4.95, a_III 2.0043 |
| 4-Methoxycatechol                    | OCH₃             | a_H² 0.55, a_H⁴ 1.0, a_III 1.15(OC₃H₅), a_III 2.0043 |
| 3,4-Dihydroxybenzylamine             | CH₂NH₂           | a_H² 0.62, a_H⁴ 0.93, a_H¹ 4.21, a_III 0.62(a_N), a_III 2.0043 |
| 3,4-Dihydroxyphenylacetic acid       | CH₂CO₂⁻          | a_H² 0.47, a_H⁴ 0.89, a_H¹ 3.47, a_III 2.0043 |
| Dopamine                             | CH₂(CH₃)COO⁻      | a_H² 0.56, a_H⁴ 0.86, a_H¹ 3.31, a_III 2.0043 |
| Dopa                                 | CH₂(CH₃)COO⁻      | a_H² 0.55, a_H⁴ 0.93, a_H¹ 2.39, a_III 1.80, a_III 2.0043 |

Table 4. Electron spin resonance data for Zn²⁺-complexed secondary o-semiquinones (indolesemiquinones) from epinephrine and its analogs.

![Chemical structure](image)

| Parent catecholamine                | R₁, R₂ in derived radical IV | Hyperfine couplings, G |
|-------------------------------------|------------------------------|------------------------|
| Epinephrine                         | H₂O H CH₃                   | a_N 4.44, a_H⁴ 5.10(3H), a_H¹ 0.15(2D), a_III 2.0040 |
| Epinephrine-α,α,β-d3⁻                | D₂O D CH₃                   | a_N 4.44, a_H⁴ 5.10(3H), a_H¹ 0.64(2H), a_III 2.0040 |
| Norepinephrine                      | H₂O H H                    | a_N 3.40, a_H⁴ 3.40(1H), a_H¹ 1.22(2H), a_III 2.0040 |
| Isoproterenol                       | H₂O H D                    | a_N 3.40, a_H⁴ 3.40(1H), a_H¹ 1.22(2H), a_III 2.0040 |
| Dihydroxynorephedrine               | H₂O H CH(CH₃)₂              | a_N 4.58, a_H⁴ 1.90(1H), a_H¹ 0.90(2H), a_III 2.0040 |

*In this structure, the other ring hydrogen also is replaced by deuterium.

Table 5. Electron spin resonance data for indolesemiquinones from photooxidation or autoxidation.

![Chemical structure](image)

| Parent compound                   | R in radical V               | Hyperfine couplings, G |
|-----------------------------------|------------------------------|------------------------|
| 5,6-Dihydroxyindole               | H                            | a_N 1.20, a_H 4.62, a_H⁴ 0.52, a_H¹ 0.64(2H), a_III 2.0041 |
| α-Methyldopa                       | CH₃                          | a_N 1.27, a_H 5.45(3H), a_H⁴ 0.55, a_H¹ 0.70, a_III 2.0041 |
Table 6. Magnetic parameters of one-electron reduced adrenochrome-metal ion complexes.

| Radical   | \( a^N \) | \( a^N_{CH_3} \) | \( a^N_{CH_2} \) | \( a^H_{CH_3} \) | \( a^H \) | \( a^H_1 \) | \( g \) |
|-----------|------------|------------------|------------------|------------------|--------|--------|-------|
| VI—Mg\(^{2+}\) | 5.36 | 5.70 | 4.78 | 5.75 | 3.45 | 0.62 | 0.38 | 2.0038 |
| VI—Zn\(^{2+}\) | 5.62 | 5.88 | 5.35 | 6.00 | 3.84 | 0.51 | 0.23 | 2.0035 |

We verified in photolysis experiments that reactions of phenoxy radicals with catechols, and of semiquinones with ascorbate, indeed occur. Formation of semiquinone is promoted by the presence of phenols, showing the ability of phenoxy radical to oxidize catechols (Fig. 13). In contrast, semiquinone concentrations are strongly quenched by ascorbate and thiols. Radical decay is pseudo-first-order, indicating a direct reaction between the semiquinone and the reducing agent.

Although reactions between these primary o-semiquinones and oxygen have been postulated, we did not observe an increased oxygen consumption above background levels from autoxidation during peroxidatic oxidation of catechols. Since the o-semiquinone is an obligate intermediate in the peroxidase system, significant electron transfer to oxygen can therefore be ruled out.

Figure 9. ESR spectra of (A), the uncomplexed semiquinone from 2-hydroxyestradiol obtained by autoxidation; (B) Zn\(^{2+}\)-complexed semiquinones from HRP/H\(_2\)O\(_2\) oxidation of 2-hydroxyestradiol; (C) Mg\(^{2+}\)-complexed semiquinones from the hydroxylation/oxidation of \( \beta \)-estradiol by tyrosinase/O\(_2\); (D) Mg\(^{2+}\)-complexed semiquinones from the hydroxylation/oxidation of 5a-hydroxyestradiol by tyrosinase/O\(_2\). In spectrum C, radicals from 2- and 4-hydroxyestradiol are denoted by (x) and (O), respectively.

Figure 10. Enzymatic oxidation of \( \beta \)-estradiol.

Figure 11. Effect of hydrogen peroxide on the duration of the steady state in radical concentration. (a) Steady state for o-semiquinone radicals from horseradish peroxidase-catalyzed oxidation of norepinephrine. Abscissa: reaction time; ordinate: ESR signal amplitude (proportional to free radical concentration). Conditions: 6mM norepinephrine, 140 mM H\(_2\)O\(_2\), 28 nM horseradish peroxidase, 227 mM Zn\(^{2+}\) in acetic acid-acetate buffer, pH 5.0. The duration of the steady state, \( t_{ss} \), is proportional to the initial hydrogen peroxide concentration. Individual measurements of \( t_{ss} \) were reproducible to \( \pm \) 10%. (b) Dependence of the duration of the steady state in semiquinone radical concentration on the initial hydrogen peroxide concentration [H\(_2\)O\(_2\)] for the three peroxidase substrates: (o) dopamine, (A) norepinephrine, and (□) dopa. Conditions: 6 mM substrate, 28 mM horseradish peroxidase, 227 mM Zn\(^{2+}\) in acetic acid-acetate buffer, pH 5.0.
Table 7. Electron spin resonance data for o-semiquinones from catechol estrogens.

| Starting material | Generating system* | Radical detected | M⁺⁺ | Hyperfine splittings, G |
|-------------------|--------------------|------------------|-----|------------------------|
| 2-Hydroxyestradiol| A                  | VII (R = H)      | Zn²⁺ | α_C=6  α_C=6  α_H=6  α_H=6₂ʰᵃʳᵐᵃᵦⁱᶜ  g |
|                   |                    |                  | 8.60 | 5.70  9.40  0.3  2.0039 |
|                   | B                  | VII (R = H)      | Mg²⁺ | 8.50  5.75  9.40  2.0042 |
| β-Estradiol       | C                  | VII (R = H)      | Mg²⁺ | 7.70  4.88  8.08  0.3,0.73  2.0044 |
| 6α-Hydroxyestradiol| C                 | VII (R = H)      | Mg²⁺ | 7.00  9.40  4.00  2.0042 |
|                   |                    | VIIa (R = H)     | Zn²⁺ | 8.60  9.00  4.00  2.0042 |
|                   |                    | VI (R = H)       | Zn²⁺ | 7.00  9.40  2.0039 |

* A = HRP-H₂O₂, pH 5.0; B = autoxidation (1 M NaOH); C = tyrosinase-O₂, pH 7.4.

Figure 12. Induction time for detection of semiquinones: (a, b, c) effect of ascorbate on the level of semiquinone radical detected during horseradish peroxidase-H₂O₂ oxidation of norepinephrine. Abscisca: reaction time; ordinate: ESR signal amplitude (proportional to free radical concentration). The time at which enzyme was added is indicated by the arrows. Individual measurements of Δt were reproducible to ± 10%. Conditions: 6 mM norepinephrine, 28 nM horseradish peroxidase, 0.28 mM H₂O₂, 227 mM Zn²⁺ in acetic acid-acetate buffer, pH 5.5. The lag in semiquinone detection that is observed is proportional to the initial concentration of ascorbate (d).

Figure 13. Effects of phenol and ascorbate on concentrations of spin-stabilized o-semiquinones from catechol: (a) 0.1 mM catechol; (b) 0.1 mM catechol + 10 mM phenol; (c) 20 mM catechol; (d) 20 mM catechol + 0.2 mM ascorbate. Radical generation was by UV photolysis of solutions in acetic acid acetate buffer, pH 5.5, containing 0.2 M Zn²⁺. In (a), (c), and (d) the majority of the absorbed light is absorbed by catechol; in (b) the majority of the absorbed light is absorbed by phenol.

Since semiquinones also can catalyze the direct reduction of H₂O₂, the feasibility of reduction of H₂O₂ by o-benzo-semiquinone and p-semiquinone (e.g., daunorubicin semiquinone) was compared (95). We were able to demonstrate this reaction for daunorubicin semiquinone, but not for o-benzo-semiquinone. While o-semiquinones derived from simple catechols do not, there-


Generation, Identification and Reactions of o-Quinones

Melanogenesis (i.e., the production of melanin) involves at least three types of o-quinones (dopapquinone, dopachrome and 5,6-indole quinone) (96-98). Each of these o-quinones possesses a characteristic visible absorption spectrum. Based on visible absorption characteristics, o-quinones from catecholamines in general can be classified as catecholamine o-quinone, aminochrome, and aminochrome or topoquinones (Tables 8-10).

o-Quinones are formed directly from two-electron oxidation of catechol(amine) by tyrosinase or from one-electron oxidation (of catechol(amine)) by HRP/H₂O₂. Whereas aminochromes are formed via intramolecular cyclization of catecholamine o-quinone (dopachrome from o-dopaquinone, adrenochrome from epinephrine o-quinone), the aminoquinones and topoquinones are formed by the addition of nitrogenous compounds and water, respectively, to o-quinones (66, 107-109).

Quinones are electrophilic in nature (Fig. 2). They undergo addition reactions with the sulfhydryl groups present in DNA polymerase and also cause the inactivation of the enzyme (18,58). Indeed the selective toxicity of melanin precursors against melanoma cells has been shown to be due to formation of o-quinones (18). Production of pheomelanin involves the addition of cysteine to o-dopaquinone followed by subsequent polymerization (64). Catecholamine o-quinones have been shown to be more toxic to melanocytes than are aminochromes (probably due to lack of electrophilic reactive sites in the latter) (18). Reactions between o-quinones and amino acids (leading to “melanoproteins”) form the

| Parent catechol(amine) | R in quinone VIII | λ_{max}, nm | Extinction coefficient, M⁻¹ cm⁻¹ | Reference |
|------------------------|-------------------|-------------|----------------------------------|-----------|
| Catechol               | H                 | 300,390     | ε_{300} = 1834                   | (97-99)   |
| 4-Methylcatechol       | CH₃               | 300,400     | ε_{300} = 2040                   | (99)      |
| 4-Tert-butylcatechol   | C(CH₃)₃           | 420         |                                 | (100)     |
| Dopa                   | CH₃CH(NH₂)CO₂     | 278,385     | ε_{390} = 1250                   | (101)     |
| Dopamine               | CH₃CH₂NH₂         | 305,394     |                                 | (18, 102, 103) |
| Norepinephrine         | CH(OH)CH₂NH₂      | 296,384     |                                 | (108)     |
| Epinephrine            | CH(OH)CH₂NHCH₃   | 302,387     |                                 | (103)     |
| N-Acetyldopamine       | CH₂CH₂NHCO₂      | 392         | ε_{392} = 1300                   | (109)     |

| Parent catechol(amine) | R in quinone | Form | λ_{max}, nm | Extinction coefficient, M⁻¹ cm⁻¹ | Reference |
|------------------------|--------------|------|-------------|----------------------------------|-----------|
| 1,24-Trihydroxybenzene | H            | p    | 485         | ε_{485} = 2042                   | (104)     |
| 4-Tert-butylcatechol   | C(CH₃)₃      | p    | 480         |                                 | (100)     |
| 6-Hydroxymelaquinone (TOPA) | CH₃CH(NH₂)COO⁻ | p    | 495         | ε_{495} = 2000                   | (102)     |
| 6-Hydroxydopa (TOPA)   | CH₃CH(NH₂)COO⁻ | o    | 465         |                                 | (102)     |
| 6-Hydroxydopa          | CH₃CH₂NH₂     | p    | 495         | ε_{495} = 2200                   | (105, 106) |

| Parent catechol(amine) | R₁, R₂, R₃ in aminochrome | λ_{max}, nm | Extinction coefficient, M⁻¹ cm⁻¹ | Reference |
|------------------------|---------------------------|-------------|----------------------------------|-----------|
| Adrenochrome           | OH H CH₃                 | 480         | ε_{480} = 4020                   | (92)      |
| Noradrenochrome        | OH H H                   | 297,477     |                                 | (103)     |
| Dopachrome             | H CO₂ H                  | 302,475     | ε_{475} = 3600                   | (97, 102) |
| Dopaminochrome         | H H H                    | 308,479     |                                 | (103)     |
basis of “browning reactions” that occur in fruits and vegetables (109).

This work was supported by National Institutes of Health grants GM-29035 and RR-01008.

REFERENCES

1. Schmelz, L., Tosk, J., Jacobs, G. and Hoffman, D. Redox potential and quinone content of cigarette smoke. Anal. Chem. 49: 1924–1929 (1977).
2. Pryor, W. A., Hales, B. J., Premovic, P. I., and Church, D. F. The nature of the free radicals in cigarette tar and suggested physiological implications. Science 220: 425–427 (1983).
3. Hecht, S. S., Carmella, S., Mori, H., and Hoffmann, D. A study of tobacco carcinogenesis. XX. Role of catechol as a major cocarcinogen in the weakly acidic fraction of smoke condensate. J. Natl. Cancer Inst. 66: 163–189 (1981).
4. Wick, M. M., Rosowsky, A., and Ratliff, J. Antitumor effects of L-glutamic acid dihydroxyanilides against experimental melanoma. J. Invest. Dermatol. 74: 112–114 (1980).
5. Riley, P. A., Morgan, B. D. G., O’Neill, T., Dewey, D. L., and Galpine, A. R. Treatment of malignant melanoma with 4-hydroxyanisole. In: Free Radicals, Lipid Peroxidation and Cancer (D. C. H. McBrien and K. Slater, Eds.), Academic Press, New York, 1982, pp. 421–438.
6. Wick, M. M. L-Dopa methyl ester as a new antitumor agent. Nature 269: 512–513 (1977).
7. Wick, M. M. Dopamine: A novel antitumor agent active against B-16 melanoma in vivo. J. Invest. Dermatol. 71: 163–164 (1978).
8. Wick, M. M. 3,4-Dihydroxybenzylamine: a dopamine analog with enhanced antitumor activity against B16 melanoma. J. Natl. Cancer Inst. 63: 1465–1467 (1979).
9. Fujita, K., Ito, S., Inoue, S., Yamamoto, Y., Takeuchi, J., Samoto, M., and Nagatsu, T. Selective toxicity of 5-S-cysteinyl-dopa, a melanin precursor to tumor cells in vitro and in vivo. Cancer Res. 40: 2545–2555 (1980).
10. Elkington, S. G., Schreiber, W. M., and Conn, H. O. Hepatic injury caused by L-alpha-methyltyrosine. Circulation 40: 589–595 (1969).
11. Yates, J. C., and Dhalia, N. S. Induction of necrosis and failure in the isolated perfused rat heart with oxidized isoproterenol. J. Mol. Cell Cardiol. 7: 907–916 (1975).
12. Gottlieb, A. J., and Wurzel, H. A. Protein-quinone interaction: In vitro induction of indirect antiglutamine reactions with methyldopa. Blood 43: 85–97 (1974).
13. Magnus, I. A. In: Dermatological Photobiology, Blackwell, London, 1976, p. 214.
14. Hochstein, P., and Cohen, G. The cytotoxicity of melamin precursors. Ann. N. Y. Acad. Sci. 100: 876–886 (1965).
15. Dybing, E., Nelson, S. D., Mitchell, J. R., Sasame, H. A., and Gillette, J. R. Oxidation of alpha-methyltyrosine and other catechols by cytochrome P-450-generated superoxide anion: possible mechanism of methyldopa hepatitis. Mol. Pharmacol. 12: 911–920 (1976).
16. Graham, D. G., Tiffany, S. M., Bell, W. R., Jr., and Gutknecht, W. F. Autoxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine, and related compounds toward C3H300 neuralblastoma cells in vitro. Mol. Pharmacol. 14: 644–653 (1978).
17. Rotman, A., Daly, J. W., and Creveling, C. R. Oxygen-dependent reaction of 6-hydroxydopamine, 5,6-dihydroxytryptamine and related compounds with proteins in vitro: a model for cytotoxicity. Mol. Pharmacol. 12: 887–899 (1976).
18. Graham, D. G., Tiffany, S. M., and Vogel, S. F. The toxicity of melamin precursors. J. Invest. Dermatol. 70: 113–116 (1978).
19. Singal, P. K., Kapur, N., Dhillon, K. S., Beamish, R. E., and Dhalia, N. S. Role of free radicals in catecholamine-induced cardiomyopathy. Can. J. Pharmacol. 69: 1390–1397 (1982).
20. Mason, H. S. Comparative biochemistry of the phenolease complex. Adv. Enzymol. 16: 105–184 (1955).
21. Howard, J. A. Absolute rate constants for reactions of oxy rad-
Electron spin resonance spectra of some ortho-monobenzoquinones and secondary radicals derived therefrom. J. Chem. Soc. 1964: 1488–1494.

45. Adams, M., Blois, M. S., Jr., and Sands, R. H. Paramagnetic resonance spectra of some semiquinone free radicals. J. Chem. Phys. 28: 774–776 (1958).

46. Felix, C. C., and Sealy, R. C. Electron spin resonance characterization of radicals from 3,4-dihydroxyphenylalanine: Semiquinone anions and their metal chelates. J. Am. Chem. Soc. 103: 2831–2836 (1981).

47. Scheulen, M., Wollenberg, P., Bolt, H. M., Kappus, H., and Remmer, H. Irreversible binding of dopa and dopamine metabolites to proteins by rat liver microsomes. Biochem. Biophys. Res. Commun. 66: 1396–1400 (1975).

48. Nelson, S. D., Mitchell, J. R., Dybing, E., and Sasase, H. A. Cytochrome P-450-mediated oxidation of 2-hydroxyestrogens to reactive intermediates. Biochem. Biophys. Res. Commun. 70: 1157–1165 (1976).

49. Sasase, H. A., Ames, M. M., and Nelson, S. D. Cytochrome P-450 and NADPH cytochrome c reductase in rat brain: formation of catechols and reactive catechol metabolites. Biochem. Biophys. Res. Commun. 78: 919–926 (1977).

50. Metzler, M., and McLachlan, J. A. Oxidative metabolism of diethyldithibroil and steroidal estrogens as a potential factor in their fetotoxicity. In: Role of Pharmacokinetics in Prenatal and Perinatal Toxicology (D. Neubert, H. J. Merker, H. Nau, and J. Langman, eds.) George Thieme, Stuttgart, pp. 156–164, 1978.

51. Degen, G. H., Eling, T. E., and McLachlan, J. A. Oxidative metabolism of diethyldithibroil in prostaglandin synthetase. Cancer Res. 42: 919–923 (1982).

52. Jones, D. P., Meyer, D. B., Andersson, B., and Orrenius, S. Conversion of catalase to the secondary-catalase-peroxide complex (compound 11) by α-methyladip. Molec. Pharmacol. 20: 159–164 (1981).

53. Iyaghi, T., and Yamazaki, I. One-electron transfer reactions in biochemical systems. III. One-electron reduction of quinones by microsomal flavin enzymes. Biochim. Biophys. Acta 172: 570–581 (1969).

54. Iyaghi, T., and Yamazaki, I. One-electron transfer reactions in biochemical systems. V. Difference in the mechanism of quinone reduction by the NADH dehydrogenase and the NAD(P)H dehydrogenase (DT-diaphorase). Biochim. Biophys. Acta 216: 282–284 (1970).

55. Nakamura, S. T., and Yamazaki, I. One-electron transfer reactions in biochemical systems. IV. A mixed mechanism in the reaction of milk xanthine oxidase with electron acceptors. Biochim. Biophys. Acta 189: 29–37 (1969).

56. Mason, H. S., and Peterson, E. W. The reaction of quinones with protamine and nucleoprotamine: N-terminal proline. J. Biol. Chem. 239: 2121–2125 (1964).

57. Byck, J. S., and Dawson, C. B. Assay of protein-quinone coupling involving compounds structurally related to the active principle of poison ivy. Anal. Biochem. 25: 123–125 (1968).

58. Wick, M. M., and Fitzgerald, G. Inhibition of reverse transcription by thymosin generated quinones related to levodopa and dopamine. Chem. Biol. Interact. 38: 99–107 (1981).

59. Tsao, W. C. Quinones formed in plant extracts. Their reaction with amino acids and peptides. Biochem. J. 112: 609–616 (1966).

60. Ito, S., and Fujita, K. Formation of cystine conjugates from dihydroxyphenylalanine and its 5-cysteinyl derivatives by peroxidase-catalyzed oxidation. Biochim. Biophys. Acta 672: 151–157 (1981).

61. Heikkila, R. E., and Cohen, G. Cytotoxic aspects of the interaction of ascorbic acid with alloxa and 6-hydroxydopamine. Ann. N. Y. Acad. Sci. 258: 221–230 (1975).

62. Heikkila, R. E., and Cohen, G. 6-Hydroxydopamine: evidence for superoxide radical as an oxidative intermediate. Science 181: 456–457 (1973).

63. Cohen, G. The generation of hydroxyl radicals in biological systems. Toxicological aspects. Photochem. Photobiol. 28: 669–675 (1978).

64. Prota, G. Recent advances in the chemistry of melanogenesis in mammals. J. Invest. Dermatol. 75: 122–127 (1980).

65. Felbeck, G. T., Jr. Structural hypotheses of soil humic acids. Soil Sci. 3: 42–48 (1971).

66. Mason, D. N., and Peterson, E. W. Melanoproteins. I. Reactions between enzyme-generated quinones and amino acids. Biochim. Biophys. Acta 111: 134–146 (1965).

67. Sinjal, P. K., Yates, J. C., Beamish, R. E., and Dhalia, W. Influence of reducing agents on adrenochrome-induced changes in the heart. Arch. Pathol. Lab Med. 105: 664–669 (1981).

68. Powis, G. Hepatic microsomal metabolism of epinephrine and adrenochrome by superoxide-dependent and independent pathways. Biochem. Pharmacol. 28: 83–89 (1979).

69. Svingen, A. B., and Powis, G. Pulse radiolysis studies of antitumor quinones: Radical lifetimes, reactivity with oxygen, and one-electron reduction potentials. Arch. Biochem. Biophys. 209: 119–126 (1981).

70. Felix, C. C., Reszka, K., and Sealy, R. C. Free radicals from photoreduction of hemoglobin in aqueous solution. Photochem. Photobiol. 37: 141–147 (1983).

71. Felix, C. C., and Sealy, R. C. o-Benzosemiquinone and its metal chelates. Electron spin resonance investigation of radicals from the photolysis of catechol in the presence of complexing metal ions. J. Am. Chem. Soc. 104: 1555–1560 (1982).

72. Planchelet, D., and Zelensky, A. V. Proton-triggered complex formation: Radical complexes of p-benzoquinone, dopa, dopamine and adrenaline formed by electron transfer reaction from excited tris (2,2′-bipyridyl)-ruthenium(II). Helv. Chim. Acta 65: 1929–1940 (1982).

73. Kalyanaraman, B., and Sealy, R. C. Electron spin resonance spin stabilization in enzymatic systems: detection of semiquinones produced during peroxidatic oxidation of catechols and catecholamines. Biochem. Biophys. Res. Commun. 106: 1119–1125 (1982).

74. Felix, C. C., and Sealy, R. C. Photolysis of melatonin precursors. Formation of semiquinone radicals and their complexation with diamagnetic metal ions. Photochem. Photobiol. 34: 423–429 (1981).

75. Kalyanaraman, B., Felix, C. C., and Sealy, R. C. Electron spin resonance spin stabilization of semiquinones produced during oxidation of epinephrine and its analogues. J. Biol. Chem. 259: 354–358 (1984).

76. Sealy, R. C., Puzyna, W., Kalyanaraman, B., and Felix, C. C. Identification by electron spin resonance spectroscopy of free radicals produced during autoxidative melanogenesis. Biochem. Biophys. Acta 800: 269–276 (1984).

77. Nilges, M. J., Swartz, H. M., and Riley, P. A. Identification by electron spin resonance of free radicals formed during the oxidation of 4-hydroxyanisole catalyzed by tyrosinase. J. Biol. Chem. 259: 2446–2451 (1984).

78. Adams, R. N., Murrell, E., McCrerey, R., Blank, L., and Karolczak, 6-Hydroxydopamine, a new oxidation mechanism. Eur. J. Pharmacol. 17: 287–292 (1972).

79. Perez-Reyes, E., and Mason, R. P. Electron spin resonance study of the autoxidation of 6-amidinodopamine. Mol. Pharmacol. 18: 594–597 (1980).

80. Pedersen, J. A., and Ollgaard, B. Phenolic acids in the genus lycopydium. Biochem. Syst. Ecol. 10: 3–9 (1982).

81. Kalyanaraman, B., Felix, C. C., and Sealy, R. C. Photoionization of melatonin precursors: an electron spin resonance investigation using the spin trap 5,5-dimethyl-1-pyrroline-1-oxide (DMPO). Photochem. Photobiol. 38: 5–12 (1982).

82. Misra, H. P., and Fridovich, I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247: 3170–3175 (1972).

83. Gee, P., and Davison, A. J. 6-Hydroxydopamine does not reduce molecular oxygen directly, but requires a co-reductant. Arch. Biochem. Biophys. 231: 164–168 (1984).

84. Symonds, M. A., and Nalbandyan, R. M. Generation of superoxide radicals in alkaline solutions of hydrogen peroxide and the effect of superoxide dismutase on this system. Biochem. Biophys. Acta 583: 279–286 (1979).
86. Yamazaki, I. Free radicals in enzyme-substrate reactions. In: Free Radicals in Biology, Vol. III, (W. A. Pryor, Ed.), 1977, pp. 183–218.

87. Eaton, D. R. Complexing of metal ions with semiquinones. An electron spin resonance study. Inorg. Chem. 3: 1268–1271 (1964).

88. Stegmann, H. B., Bergler, H. U., and Scheffler, K. "Spin-stabilization" via complex formation; ESR investigation of some catecholamine semiquinones. Angew. Chem. Int. Ed. 20: 389–390 (1981).

89. Kalyanaraman, B., Felix, C. C., and Sealy, R. C. Peroxidatic oxidation of catecholamines: a kinetic electron spin resonance investigation using the spin stabilization approach. J. Biol. Chem. 259: 7584–7589 (1984).

90. Wertz, J., Reitz, D. and Dravnieks. In: Free Radicals in Biological Systems (M. S. Blois, Jr., Ed.), Academic Press, New York, 1961, pp. 182–193.

91. Borg, D. C., and Elmore, J. J., Jr. Evidence for restricted molecular conformation and for hindered rotation of side chain groups from EPR of labile free radicals. In: Magnetic Resonance in Biological Systems (A. Ehrenberg, B. G. Malmstrom, and T. Vanngard, Eds.), Pergamon Press, Oxford, 1967, pp. 341–349.

92. Borg, D. C. Transient free radical forms of hormones: EPR spectra from catecholamines and adrenochrome. Proc. Natl. Acad. Sci. (U.S.) 53: 633–638 (1965).

93. Green, S., Mazur, A. and Shorr, E. Mechanism of the catalytic oxidation of adrenaline by ferritin. J. Biol. Chem. 220: 237–255 (1956).

94. Kalyanaraman, B., Sealy, R. C., and Sivarajah, K. An electron spin resonance study of o-semiquinones formed during the enzymatic and autoxidation of catechol estrogens. J. Biol. Chem. 259: 14018–14022.

95. Borg, D. C., Schaich, K. M., Elmore, J. J., Jr., and Bell, J. A. Cytotoxic reactions of free radical species of oxygen. Photochem. Photobiol. 28: 887–907 (1978).

96. Kalyanaraman, B., Sealy, R. C., and Sinha, B. K. An electron spin resonance study of the reduction of peroxides by anthracine semiquinones. Biochim. Biophys. Acta 799: 270–275 (1984).

97. Sealy, R. C., Felix, C. C., Hyde, J. S., and Swartz, H. M. Structure and reactivity of melanins: influence of free radicals and metal ions. In: Free Radicals in Biology (W. A. Pryor, Ed.), Vol. IV, 1980, pp. 209–259.

98. Mason, H. S. The chemistry of melanin III. Mechanism of the oxidation of dihydroxyphenylalanine by tyrosinase. J. Biol. Chem. 172: 83–99 (1948).

99. Mason, H. S. The chemistry of melanin. VI. Mechanism of the oxidation of catechol by tyrosinase. J. Biol. Chem. 181: 809–811 (1949).

100. Duckworth, H. W., and Coleman, J. E. Physiochemical and kinetic properties of mushroom tyrosinase. J. Biol. Chem. 245: 1613–1625 (1970).

101. Menter, J. M., and Willis, I. The interaction of L-dopa with p-tet-butylcatechol. J. Invest. Dermatol. 75: 257–260 (1980).

102. Garcia-Carmona, F., Garcia-Canoas, F., Iborra, J. L., and Lozano, J. A. Kinetic study of the pathway of melanization between L-dopa and dopachrome. Biochim. Biophys. Acta 717: 124–131 (1982).

103. Graham, D. G., and Jeffs, P. W. The role of 2,4,5-trihydroxyphenylalanine in melanin biosynthesis. J. Biol. Chem. 252: 5729–5734 (1977).

104. Graham, D. Oxidative pathways for catecholamines in the genesis of neuromelanin and cytotoxic quinones. Mol. Pharmacol. 14: 633–643 (1978).

105. Corbett, J. F. The chemistry of hydroxy-quinones. Part VI. Formation of 2-hydroxysemiquinones during the autoxidation of benzene-1,2,4-triols in alkaline solution. J. Chem. Soc. (C) 1970: 2101–2106.

106. Swan, G. A. Studies related to the chemistry of melamins. Part XIV. The alleged formation of a p-quinonoid aminochrome by oxidation of 2,4,5-trihydroxyphenylethylamine. J. Chem. Soc. Perkin Trans. 1: 339–341 (1976).

107. Senoh, S., and Witekop, B. Formation and rearrangements of aminochromes from a new metabolite of dopamine and some of its derivatives. J. Am. Chem. Soc. 81: 6231–6240 (1959).

108. Hawley, M. D., Tatawawadi, S. V., Pierkarski, S., and Adams, R. N. Electrochemical studies of the oxidation pathways of catecholamines, J. Am. Chem. Soc. 89: 447–450 (1967).

109. Mason, H. S. Structures and functions of the phenolase complex, Nature 177: 79–81 (1956).

110. Hurrell, R. F., and Finot, P. A. Nutritional consequences of the reactions between proteins and oxidized polyphenolic acid. Adv. Exp. Med. Biol. 177: 423–435 (1984).