Some Reactions and Properties of Nitro Radical-Anions Important in Biology and Medicine

by Peter Wardman*

Nitroaromatic compounds, ArNO₂, have widespread actual or potential use in medicine and cancer therapy. There is direct proof that free-radical metabolites are involved in many applications, and an appreciation of the conceptual basis for their therapeutic differential; however, an understanding of the detailed mechanisms involved is lacking. Redox properties control most biological responses of nitro compounds, and the characteristics of the one-electron couple: ArNO₂/ArNO₂⁻ are detailed. The “futile metabolism” of nitroaryl compounds characteristic of most aerobic nitroreductase systems reflects competition between natural radical-decay pathways and a one-electron transfer reaction to yield superoxide ion, O₂⁻. Prototropic properties control the rate of radical decay, and redox properties control the rate of electron transfer to O₂ or other acceptors. There are clear parallels in the chemistry of ArNO₂⁻ and O₂⁻. While nitro radicals have frequently been invoked as damaging species, they are very unreactive (except as simple reductants). It seems likely that reductive metabolism of nitroaryl compounds, although generally involving nitro radical-anions as obligate intermediates (and this is required for therapeutic selectivity towards anaerobes), results in biological damage via reductive metabolites of higher reduction order than the one-electron product.

Introduction

Although the other papers in this issue attest to the widespread interest in free-radical intermediates in the action of several classes of medically important compounds, it is arguable that nitrocompounds are the one class of drug in which direct proof of radical production in intact target organisms has been demonstrated (1–3) and in which the free-radical reaction almost certainly responsible for the therapeutic selectivity has been observed directly (4). The combination of identification and measurement of steady-state concentrations of radicals by electron spin resonance (ESR) and spectrophotometric monitoring of reaction kinetics following radical generation by pulse radiolysis (5) has provided considerable insight into the most important reactions and properties of nitro radical-anions important in biology and medicine.

Mason and colleagues (1–3,6–16) obtained high resolution ESR spectra of steady-state concentrations of nitro radical-anions of a variety of medically-important drugs in biological preparations:

ArNO₂ + “nitroreductases” → ArNO₂⁻ (1)

and also established the concept (7,8) of “futile” reduction or metabolism in aerobic conditions, where back-oxidation occurs (4,17):

ArNO₂⁻ + O₂ → ArNO₂ + O₂⁻ (2)

in competition with other one-electron transfer reactions to appropriate electron-acceptors and with the “natural” decay pathways such as disproportionation (4,9,18–21):

2 ArNO₂⁻ → ArNO₂ + ArNO₂²⁻ (= ArNO) (3)

In this paper we restrict ourselves to considering nitroaromatic compounds, ArNO₂⁺ in the absence of, e.g., substituents with basic functions, the one-electron adducts ArNO₂⁻ are normally radical-anions at physiological pH because the pKₐ for the dissociation:

ArNO₂H ≡ ArNO₂⁻ + H⁺ (4)

is usually << 7 (20,22–24). However, pH and pKₐ generally defines the rate of reaction (3) (18,21,24), and these properties are important in other electron-transfer reactions.

Although there may be little or no net nitroxidation in aerobic systems because of reaction (2), there may be a stimulation of respiration, a feature which Biaglow, Durand, Sutherland, et al. (25–29) recognized may be

*Gray Laboratory of the Cancer Research Campaign, Mount Vernon Hospital, Northwood, Middx. HA6 2RN, England.
important in the potentially widespread use of nitroaryl compounds in cancer therapy (31–34). The oxygen tension in tumor cells may control the radiotherapeutic response, and nitro compounds (and other oxidants) are able to sensitize hypoxic cells to radiation, with a negligible effect on the radiosensitivity of well-oxygenated tissues. In addition to this application, there is considerable interest in combination chemotherapy involving nitro compounds, or “chemosensitization” (33,34).

There is currently much interest (35) in the enzyme, superoxide dismutase and the possible toxic properties of $O_2^-$, or, more plausibly, subsequent products obtained via metal-catalyzed Fenton-type chemistry (36) and in the invoking of “redox cycling” (37) [reactions (1) and (2)] in the analogous chemistry with anthracyclines and other quinones. Since the flux of $O_2^-$ is stimulated by most nitro compounds via reaction (2), one might have expected the biological properties of nitro compounds to reflect possible “superoxide” toxicity. However, most nitro compounds are less toxic towards mammalian cells in air than in hypoxia (38–40), which must reflect the much more damaging, competing reactions such as (3) and subsequent reductive pathways. The details of the mechanism of toxicity of antiparasitic nitro compounds is the subject of an article by Moreno and Docado in this issue (41), and the present paper is restricted to the more basic chemical properties of nitro radical-ions which control the rate of reactions (1)–(3) and hence their biological properties.

Nitromidazoles are the class of nitro compounds most widely used in medicine, and the review of Josephy and Mason (42) covers the literature on the reduction products of nitromidazoles to 1982. More recent work has provided further information, e.g., on the instability of reduction intermediates (43,44) and the reaction of reductive fragments with glutathione (45), nucleosides (46), and protein (47,48). Although there is much emphasis on higher reduction, the nitro radical-ions is thought to be the obligatory intermediate involved in nitro-reduction by most (but not all) organisms, and its radical chemistry is therefore central to the use of nitro compounds in medicine.

**Redox Properties of Nitroaromatic Compounds**

Most biological properties of nitroaryl compounds reflect the ease of nitro-reduction in a remarkably similar way (28,49,50), although the apparent simplicity of most redox dependences is unfortunately not extended to the organisms of most interest in medicine (51–53). Hence the thermodynamic parameter characterizing the relative ease of reduction is of major importance in defining the likely biological properties of different nitro compounds; in fact, the parameter also controls the rate of reaction (2), and other reactions with electron acceptors, as well as (1). Reaction (1) involves the one-electron couple: ArNO$_2$/ArNO$_2^-$, and it is the reduction potential $E$ of this couple in water at physiological pH which is the most appropriate index of the redox properties of nitroaromatic compounds in the present context.

Since ArNO$_2^+$ is unstable in aqueous solution at pH ~ 7 (see below), conventional electrochemical measurements such as polarographic half-wave potentials, $E\%_1/2$ cannot be equated with potentials for the one-electron couple, $E$(ArNO$_2$/ArNO$_2^-$). However, electrochemical measurements of $E\%_1/2$ using, e.g., cyclic voltammetry in aprotic solvents (54) or polarography in water (55) generally parallel the thermodynamically reversible one-electron potentials in water at pH 7 such that the values are numerically similar when $E$ is expressed on the hydrogen scale (NHE) and $E\%_1/2$ on the calomel reference (SCE). Thus, generally, $E \approx (E\%_1/2 - 0.24 \text{ V})$ to a fair approximation. The higher the value (more positive), the more electron-affinic (more powerful oxidant) the nitro compound.

The most powerful and reliable method to determine $E$(ArNO$_2$/ArNO$_2^-$) is from pulse radiolysis measurements of the equilibrium constant for one-electron transfer equilibria involving ArNO$_2$ and a reference compound of known reduction potential such as a quinone or bipyridinium compound (56,57):

$$\text{ArNO}_2^- + Q \rightleftharpoons \text{ArNO}_2 + Q^-$$

(5)

since

$$\Delta E_5 = E(Q/Q^-) - E(\text{ArNO}_2/\text{ArNO}_2^-)$$

(6)

$$E(\text{ArNO}_2/\text{ArNO}_2^-) = E(Q/Q^-) - (RT/F) \ln K_5$$

(7)

$$E(\text{ArNO}_2/\text{ArNO}_2^-) = E(Q/Q^-) - 0.059 \log K_5$$

(8)

if $E$ is in volts. The yields and reactions of the species produced upon radiolysis of aqueous solutions are so well established that the design of such experiments is a matter of routine (5). The radicals: ArNO$_2^+$, Q$^-$ are usually generated within a microsecond or so of the end of a radiolysis pulse of equally short duration, and the equilibrium (5) attained and the equilibrium constant $K_5$ measured spectrophotometrically within (typically) 10–200 µsec, i.e., before the unstable radicals can decay via routes such as (3) (5,56,57).

A scale of reduction potential spanning the range appropriate for virtually all nitroaryl compounds of medical or biological interest is shown in Figure 1. The potentials of three common nitroheterocyclic pharmaceuticals are seen to be significantly lower than that of oxygen. [Note: $E$(O$_2$/O$_2^-$) is correctly given as $-0.33 \text{ V}$ vs. NHE at pH 7, since the thermodynamic standard state for oxygen is unit fugacity, i.e., 1 atmosphere pressure. Use of the Nernst relationship and converting to a nonstandard state of 1 mole/dm$^3$ O$_2$/O$_2^-$ (the same standard state as ArNO$_2$/ArNO$_2^-$) gives an effective $E$(O$_2$/O$_2^-$) of ca. $-0.15 \text{ V}$, a value more appropriate for direct comparison with $E$(ArNO$_2$/ArNO$_2^-)$ (58).

Also shown in Figure 1 are the potentials of compounds which illustrate the effects of additional, electron-withdrawing substituents in (in this case) the 2-nitromidazole ring system. Such effects can be readily
predicted once the potentials of two or three compounds 
in a given series have been measured, using predictive 
relationships based upon Hammett substituent con-
tants (59). Thus for 5(R)-1-methyl-2-nitroimidazoles:

\[ E/V = -0.406 + 0.146 \sigma_p(R) \]  
(9)

and for 4(R) - nitrobenzenes:

\[ E/V = -0.484 + 0.168 \sigma_p(R) \]  
(10)

in water at pH 7, \( \sim 298^\circ K \).

Figure 2 illustrates the variation of estimated values of 
\( E(\text{ArNO}_2/\text{ArNO}_2^-) \) (under physiological conditions) 
for some of the more common nitroaryl ring systems, 
and Table 1 lists values of \( E \) for the compounds of most 
interest in biology, medicine, and cancer therapy. [The 
author is preparing a more complete compilation of reduc-
tion potentials of couples involving free radicals in 
aqueous solutions, to be published in the U.S. National 
Standard Reference Data Series, which will give full 
details and a bibliography; most of the values shown 
have been published in a variety of references (4, 20, 
30, 49, 53, 56, 57).]

The position of the important one-electron transfer 
equilibrium (2):

\[ \text{ArNO}_2^- + \text{O}_2 \rightleftharpoons \text{ArNO}_2 + \text{O}_2^- \]  
(2)

can be simply calculated from these values of \( E \) and the

relationship:

\[ \log K_2 = \{ -0.155 - [E(\text{ArNO}_2/\text{ArNO}_2^-)/V] / 0.059 \} \]  
(11)

Table 1. Values of reduction potential and equilibrium constants 
for electron-electron transfer to oxygen for some biologically 
important nitroaryl compounds.

| Compound                  | \( E(\text{ArNO}_2/\text{ArNO}_2^-)/V \) vs. NHE | \( K_2 \) |
|---------------------------|-----------------------------------------------|--------|
| 4-Nitroquinoline-N-oxide  | -0.175                                        | 2.2    |
| Nifuripipone              | -0.214                                        | 10     |
| Furalidate                | -0.248                                        | 37     |
| Nitrofurazone             | -0.257                                        | 53     |
| Nitrofurantoin            | -0.264                                        | 70     |
| Furaspur                  | -0.338                                        | \( 1.2 \times 10^6 \) |
| 53,56                      |                                              |        |
| 54,55                      |                                              |        |
| Nitrofurazone             | -0.346                                        | \( 1.7 \times 10^4 \) |
| 4-Nitrobenzoic acid       | -0.390                                        | \( 6.4 \times 10^3 \) |
| 4-Nitrobenzoic acid       | -0.398                                        | \( 8.8 \times 10^3 \) |
| Nitrofurazone             | -0.389                                        | \( 9.1 \times 10^3 \) |
| 4-Nitrobenzoic acid       | -0.390                                        | \( 9.5 \times 10^3 \) |
| Nitrofurazone             | -0.410                                        | \( 2.1 \times 10^4 \) |
| Azomycin                  | -0.418                                        | \( 2.5 \times 10^4 \) |
| 4-Nitrobenzoic acid       | -0.425                                        | \( 3.7 \times 10^4 \) |
| Nitrofurazone             | -0.457                                        | \( 1.3 \times 10^5 \) |
| 4-Nitrobenzoic acid       | -0.464                                        | \( 1.7 \times 10^5 \) |
| Nitrofurazone             | -0.467                                        | \( 1.9 \times 10^5 \) |
| 4-Nitrobenzoic acid       | -0.475                                        | \( 2.6 \times 10^5 \) |
| Nitrofurazone             | -0.475                                        | \( 2.6 \times 10^5 \) |
| 4-Nitrobenzoic acid       | -0.486                                        | \( 4.0 \times 10^5 \) |

Figure 2. Estimated values of reduction potential, \( E(\text{ArNO}_2/ 
\text{ArNO}_2^-) \) vs. NHE in water at pH 7 of some typical 
nitroaryl systems. The examples are based upon measurements of the 
compounds or simple derivatives where R = alkyl or hydroxalkyl, 
etc., and the values may be significantly different when additional 
ring substituents are present (see Fig. 1).
Estimates of $K_2$ are included in Table 1.

The positions of other electron-transfer equilibria of interest may be calculated similarly, replacing $Q/Q^-$ by the appropriate couple in eq. (5) and using the relationship (8). Thus ascorbate, $AH^-$ has frequently been of interest as a potential electron donor to nitroaryl compounds, e.g., with 4-nitroquinoline-N-oxide (27, 60–63):

$$\text{ArNO}_2^- + AH^- \rightleftharpoons \text{ArNO}_2^- + AH^+ \quad (12)$$

The reduction potential of the ascorbyl radical at pH 7, $E(\text{AH}/\text{AH}^-)$ can be estimated reliably at 0.30 V vs. NHE from either measurements of one-electron transfer equilibria at pH 13.5 (64) or calculations (65) based upon the semiquinone formation constant from ESR measurements (66). Hence:

$$\log K_{12} = \{E(\text{ArNO}_2^-/\text{ArNO}_2^+)/(V - 0.30)\}/0.059 \quad (13)$$

and $K_{12}$ is estimated to be $9 \times 10^{-9}$ for 4-nitroquinoline-N-oxide, $3 \times 10^{-10}$ for typical 5-nitrofurans, $2 \times 10^{-12}$ for simple 2-nitroimidazoles and $5 \times 10^{-14}$ for metronidazole and analogs. These equilibria are, of course, pH-dependent since $E(\text{AH}/\text{AH}^-)$ is decreased, e.g., to 0.015 V at pH 13.5 (64), at which pH $K_{12}$ is increased to ca. $1 \times 10^{-7}$ for simple 2-nitroimidazoles.

In spite of equilibrium (12) being thermodynamically so unfavorable ($K_{12} < 10^{-8}$ even for the most electron-affinic nitro compound), ascorbate can still be a potential one-electron reductant because the products of reaction (12) are unstable and are being continuously removed by, e.g., disproportionation or reaction (3). A further reaction may also be considered. The one-electron reduction potentials for addition to a second electron to nitroaryl compounds [the reduction potential of the nitro radical-anion, $E(\text{ArNO}_2^-/\text{ArNO}_2^{2-})$ or $E(\text{ArNO}_2^{2-}/\text{ArNO}_2)$] are unknown for aqueous solutions at pH 7, although there is some evidence (see below) that $\text{ArNO}_2^{2-}$ is a poorer oxidant than $\text{O}_2^-$ at physiological pH; apparently $\text{ArNO}_2^{2-}$ is unable to oxidize the Cu(I) form of Cu-Zn superoxide dismutase, whereas $\text{O}_2^-$ is able to do so (67). However, it is not inconceivable that oxidation of $\text{AH}^-$ by $\text{ArNO}_2^{2-}$ may play a significant role; the reaction would be expected to be at least as complex as the analogous oxidation by $\text{O}_2^-$ (68). Indirect evidence for nitro radical formation from a nitrofuran with ascorbate (pH 7) as electron donor has been obtained (69), making use of a diagnostic cis-trans chain isomerization reaction (69, 70) which yields up to ~200 molecules isomerized per radical-anion produced (71) (see below):

$$\text{cis-}AF - 2 + \text{"nitreductases"} \rightarrow \text{trans-}AF - 2 \quad (14)$$

Similar considerations apply to the thermodynamics of formation of nitro radical-anions from other reductants, e.g. reduced flavin, FMNH$_2$.

$$\text{ArNO}_2^- + \text{FMNH}_2 \rightleftharpoons \text{ArNO}_2^- + \text{FMNH}^+ (H^+) \quad (15)$$

Using a value of $E(\text{FMNH}/\text{FMNH}^H) = -0.124$ V (72), we have

$$\log K_{18} = \{(E(\text{ArNO}_2^-/\text{ArNO}_2^+))/V + 0.124\}/0.059 \quad (16)$$

and $K_{18}$ is estimated to be $8 \times 10^{-6}$ for 4-nitrobenzoate at pH 7. Again, in spite of unfavorable thermodynamics, FMNH$_2$ reduces many ArNO$_2$ at readily measurable (and redox-controlled) rates (78), and FMN + NADH generates steady-state concentrations of ArNO$_2^{2-}$ which are detectable by ESR (7).

These calculations are valid only for pH 7, since the potentials of both couples may vary with pH. In the case of the couple, ArNO$_2^{2-}$, the potential $E_i$ at any pH = $i$ may be calculated from:

$$E_i = E_7 + 0.059 \log \{|K_4 + [H^+]|/K_4 + 10^{-7}\} \quad (17)$$

provided there are no additional substituents with prototropic properties. Since $pK_4 < 7$ for all known, simple nitroaryl compounds (see below), this pH dependence of $E(\text{ArNO}_2^-/\text{ArNO}_2^{2-})$ is frequently unimportant in respect of physiological conditions. However, acidic or basic substituents complicate the issue considerably (24, 56, 57, 59).

A note of caution is also appropriate concerning the rates of reactions which may have readily calculable thermodynamic parameters but which are catalyzed either by enzymes in vitro or even by simple, free metal ions. The reduction of ArNO$_2$ by flavins or thiols is catalyzed by trace quantities of Fe(II) (63, 73–76). One-electron reduction by free thiols is thermodynamically much less favorable than by ascorbate, since $E(\text{RS}^-/\text{RS}^2-)$ must be much more positive than $E(\text{AH}/\text{AH}^-)$ at pH – 7, indeed higher than $E(PZ^-/PZ) = 0.8–0.9$ V where $PZ$ = common phenothiazines (77).

**Prototropic Properties of Nitro Radical-Anions**

In addition to being of potential importance in defining the pH-dependence of redox properties, prototropic equilibria control the natural lifetimes of nitroaryl radical-anions in aqueous solution. Equilibrium (4) is written as a dissociation of an oxygen acid, but protonation at sites other than oxygen may be important, so that Eq. (18) may be considered a general representation, of which Eq. (4) is a specific example.

$$\text{ArNO}_2^-H^+ \rightleftharpoons \text{ArNO}_2^- + H^+ \quad (18)$$

Grünbein et al. (22) estimated the values of $pK_a (pK_{18})$ of 17 nitrobenzene derivatives from the pH-dependent absorption spectra of the radicals produced upon one-electron reduction by pulse radiolysis. The measurements of $pK_a$ spanned the range 2.2 (1,2-dinitrobenzene) to 3.9 (1-ethoxy-2-nitrobenzene); since only one derivative had any other prototropic functions (3-nitroben-
zoic acid), the values must represent protonation at oxygen, equilibrium (4). The effects of different substituents upon \( pK_a \) for nitrobenzene (18) correlated well with Hammett \( \sigma \) substituent constants, in turn reflecting the spin density on the nitro group in the radical (58). Published estimates of \( pK_a \) for 12 substituted nitrobenzenes (18,20,22,78), together with values of \( E \) at pH 7 (20,56,73,79) yields:

\[
pK_4 = (0.61 \pm 0.23) - (5.2 \pm 0.6)(E/N) \quad (19)
\]

This relationship, if it were applicable to heterocycles such as 5-nitrofurans or nitroimidazoles, would predict values of \( pK_a \) in the region of \(<2\), ca. 2.7 and 3.2 for typical 5-nitrofurans, 2- and 5-nitroimidazoles respectively. Values of \( pK_a \) as low as 1–1.2 for 5-nitrofurans have been reported (23), but prototropic dissociations with \( pK \) = 5.7 or 6.1 were reliably characterized for the nitroimidazoles, misonidazole, and metronidazole, respectively (24). These latter values must reflect protonation on the unsubstituted imidazolyl nitrogen in these molecules and not on the nitro group, a view supported by the symmetrical ESR hyperfine pattern recorded on the unsubstituted 2-nitroimidazole (azomycin) radical-anion (80).

Thus even simple nitroimidazole radicals have two sites for protonation in the readily-accessible range of pH: NO\(_2\) oxygen and ring nitrogen. When ring substituents carry groups with prototropic properties, e.g., nitrogenous bases or carboxylic acids, the \( pK_a \) for dissociation of these additional proton sites may be significantly different in the ground state and radical even when, e.g., the side-chain nitrogen is “insulated” from the nitroaryl ring by a 2- or 3-carbon aliphatic chain. A typical example in the important 2-nitro-1-imidazolylalkylamine series has been discussed (59). More dramatic shifts in \( pK_a \) between ground state and one-electron adduct are found with the protonation of the unsubstituted (N-3) imidazolyl nitrogen in some simple N-alkyl/alkanol substituted 2-, 4- and 5-nitroimidazoles, where the increase in \( pK_a \) upon electron addition is around 6.2, 5.5, and 3.6 units, respectively (24,81). ESR studies have characterized the dissociation of acidic ring N-H protons in other nitroaryl radical-anions lacking carbon substitution at nitrogen (80,82).

**Natural Lifetimes of Nitro-Radical Anions**

The normal decay pathway of most nitro radical-anions at pH ~ 7 in water is that of second-order disproportionation:

\[
2 \text{ArNO}_2^- + 2 \text{H}^+ \rightarrow \text{ArNO}_2 + \text{ArNO} + \text{H}_2\text{O} \quad (20)
\]

However, there are two major complications and at least one important exception to this rule. Firstly, the formation of the nitroso compound, ArNO occurs (at least in the case of nitrobenzene) via a hydrated form which dissociates in an acid-catalyzed reaction (18). Secondly, Eq. (20) includes the involvement of protons in the overall reactions, and there are three distinct reaction pathways which must be considered, precisely analogous to the disproportionation of \( \text{HO}_2^-/\text{O}_2^- \) to yield \( \text{H}_2\text{O}_2 \) (83):

\[
2 \text{ArNO}_2\text{H} \rightarrow \text{ArNO}_2 + \text{ArNO} + \text{H}_2\text{O} \quad (21)
\]

\[
\text{ArNO}_2\text{H}^+ + \text{ArNO}_2^- (+ \text{H}^+) \rightarrow \text{ArNO}_2 + \text{ArNO} (+ \text{H}^+) \quad (22)
\]

\[
2 \text{ArNO}_2^- (+ 2\text{H}^+) \rightarrow \text{ArNO}_2 + \text{ArNO} (+ \text{H}_2\text{O}) \quad (23)
\]

As with \( \text{O}_2^- \), it appears that \( \text{ArNO}_2^- \) does not react with itself in water at a significant rate, i.e., \( k_{22} = 0 \), except in the case of 4-nitroacetophenone (4). The kinetics then simplifies to:

\[
2k_{\text{obs}} = [2k_{21} + 2k_{22}(K_{18}/[\text{H}^+] )]/(1 + K_{18}/[\text{H}^+] )^2 \quad (24)
\]

where the rate equation is defined as:

\[
-d[\text{ArNO}_2^-]/dt = 2k_{\text{obs}} [\text{ArNO}_2^-]^2 \quad (25)
\]

and the half-life at any initial concentration \([\text{ArNO}_2^-]_0\) is given by:

\[
t_{1/2} = 1/k_{\text{obs}}[\text{ArNO}_2^-]_0 \quad (26)
\]

Figure 3 illustrates the typical, pH-dependent second-order rate constant for the decay of metronidazole radicals measured by pulse radiolysis. More extensive studies (21) using pH 7.4, isotonic ionic strength, \( \sim 298^\circ\text{K} \), have proven the radicals decay by accurate second-order kinetics with \( k_{\text{obs}} = 4.2 \times 10^8 \text{dm}^3/\text{mole-sec} \) under these conditions. The values for radicals from other 5-nitroimidazoles such as misonidazole and nimorazole were within a factor of two of that for metronidazole (21), and independent ESR observations of the disproportionate rate of 4-nitrobenzoate radicals at pH 7.4 gave a value of \( k_{\text{obs}} \) [eq. (25)] = \( 8.5 \times 10^8 \text{dm}^3/\text{mole-sec} \) (9,84). More extensive, pulse radiolysis measurements of substituted nitrobenzene radicals (20) provided estimates of \( k_{\text{obs}} \) at pH 7 in the range 7 \times 10^8 to 3.3 \times 10^8 \text{dm}^3/\text{mole-sec}, although most were \(< 10^7 \text{dm}^3/\text{mole-sec} \) (values at pH 7.4 would be expected to be 2.5 times lower than those at pH 7). The decay kinetics of some other nitroimidazole radicals (e.g., 5-chloro-1-methyl-4-nitroimidazole, \( 2k_{\text{obs}} = 1 \times 10^8 \text{dm}^3/\text{mole-sec} \) at pH 7) (81), but not 2-nitroimidazole radicals (21) are broadly similar to those illustrated in Figure 3 for metronidazole. A value for \( 2k_{\text{obs}} = 5 \times 10^7 \text{dm}^3/\text{mole-sec} \) can be considered typical for the decay of most simple nitro radicals under physiological conditions.

The steady-state concentrations of nitro radicals can
be measured by ESR and are typically of the order of 1 μmole/dm³ in many experiments (7,9). The concentrations in intact, target organisms depend on the substrate (2) and likely in vivo values are difficult to predict. However, typical natural lifetimes [with respect to decay by reaction (20) only] of nitro radicals under physiological conditions could well be as long as 20 sec [from Eq. (26), using a steady-state concentration of 1 μmole/dm³ and 2k_obs = 5 × 10⁴ dm³/mole-sec].

These calculations can not be applied to the radicals from 2-nitroimidazoles, which decay in an unknown, first-order pathway which is, nonetheless, still first-order in [H⁺]:

\[
\text{ArNO}_2^- (\pm H^+) \rightarrow \text{products} \tag{27}
\]

with \( -d[\text{ArNO}_2^-]/dt = k_{27} [\text{ArNO}_2^-] \), and \( k_{27} \) around 5–10/sec at pH 7.4 for simple 2-nitroimidazoles (21). The natural lifetimes of 2-nitroimidazoles radicals are thus independent of their steady-state concentration, within a sensible physiological range (21), and are around 0.1 sec at pH 7.4 (= ln 2/k$_{27}$).

Lifetimes of nitroimidazole radicals are many seconds at high pH (4,21,24,81), similar to the behavior of radicals from nitrobenzene and derivatives (18,19,85,86). A report (23) that the second-order rate constant for the decay of the 5-nitrofuroic acid radical anion remained essentially unchanged (2k = 2.2 × 10⁹ dm³/mole-sec) from pH 10.5 to 3.3 is completely inconsistent in both magnitude and pH-independence with numerous other observations (see below). The dependence of the steady-state concentration of radicals on the square root of the (reductase) protein concentration (3,9,10,13,16) indicates the general second-order decay pathway, Eq. (20), is common to nitrobenzenes, -furans, and -imidazoles (except 2-nitroimidazoles).

The pH-dependent lifetimes shown in Figure 3 raise an interesting point. That the transition in the value for 2k_obs for 4-nitroacetophenone radicals occurs at a pH significantly higher than pK₄ (= 2.7 (77)) reflects the relative values: \( k_{22} > k_{21} \), a similar situation to that seen with HO₂/O₂⁺ (83).

**Electron-Transfer Reactions of Nitro Radical-Anions**

The values of the couple: \( E(\text{ArNO}_2^-/\text{ArNO}_2^+) \) may be used to assess not only the thermodynamic feasibility of one-electron reduction of \( \text{ArNO}_2^- \) by any potential "nitroreductase" (flavin, ascorbate, Fe/S protein, etc.) but also the likelihood of electron donation from \( \text{ArNO}_2^- \) to potential acceptors. In the present context the most important of these is obviously oxygen [reaction, (2)].

Equation (11) defines the position of equilibrium but says nothing about the kinetics of reaction (2) in particular:

\[
\text{ArNO}_2^- + \text{O}_2 \rightarrow \text{ArNO}_2^+ + \text{O}_2^- \tag{2}
\]

Direct measurements of the kinetics of this reaction for numerous nitroaryl compounds have been made, illustrating the expected, Marcus-type redox dependence; the initial report (4) provides a typical cross section. Values of \( k_2 \) at ~298K range from about 4 × 10⁷ dm³/mole-sec for compounds with \( E = -0.6 \) V (e.g., 5-nitroorotic acid), 7 × 10⁶ dm³/mole-sec for \( E = -0.5 \) V (e.g., metronidazole), 4 × 10⁶ dm³/mole-sec for \( E = -0.4 \) V (e.g., misonidazole) to 2.3 × 10⁵ dm³/mole-sec for typical 5-nitrofurans with \( E = -0.25 \)V. The Arrhenius parameters have been measured for six typical compounds (P. Wardman and E. D. Clarke, unpublished):

\[
k = A \exp(-E_a/RT) \tag{28}
\]

with \( E_a \) all in the range 30–39 kJ/mole; the algorithms for the temperature dependences of \( k_2 \) for metronidazole and misonidazole have been reported (4,21).

There seems no question that the product of reaction (2) is indeed \( \text{O}_2^- \) with restoration of \( \text{ArNO}_2^- \). Preliminary spectral evidence that \( \text{O}_2^- \) is a product (4) is substantiated by the spectra shown in Figure 4, which are the results of repeating the earlier work (4) with further precautions taken to eliminate artefacts from scattered light (or at least, to ensure identical artefacts in the control spectrum of \( \text{O}_2^- \)). Pulse radiolysis measurements always measure the change in absorbance introduced by the conversion of ground state to radical; that the final spectrum in Figure 4 agrees with that of \( \text{O}_2^- \) requires restoration of the nitro radical to ground state,
which absorbs strongly in this spectral region. ESR spin-trapping of $O_2^{-}$ (17) provides further evidence that $O_2^{-}$ is a product.

Since an earlier report (87) indicated a rate constant for reaction of the nifuroxime radical-anion around 4 orders of magnitude higher than our value (4) (and about 50-fold higher than that for any other nitro compound so far reported, let alone for a compound with high electron affinity, i.e., with relatively low "driving energy"), it seems appropriate to present some raw data to justify our claim. Figure 5 shows the absorption changes produced upon generating the nitro radical in N$_2$-, air- or O$_2$-saturated solutions of formate (to scavenge H and OH) (5); tert-butanol, an alternative OH scavenger was used by Greenstock and Dunlop (87). There are rapid spectral changes in the tert-butanol system, presumably resulting from reaction of tert-butanol radicals with nifuroxime, or radical-radical reactions; the natural lifetimes of simple nitro radicals are without exception much longer than implied by the data of Greenstock and Dunlop (87). Although the first-order dependence of radical decay on oxygen concentration reported by Greenstock and Dunlop is impressive, it appears that the tert-butanol system is not satisfactory for studying reaction (2) in this instance.

Since the reduction potential, $E$ of even the most electron-affinic nitroaryl compound so far reported (4-}

\[
\text{nitroquinoline-N-oxide} \quad \text{is} \quad -0.18 \text{ V, it is not surprising that electron transfer from nitro radicals to more powerful oxidants such as Fe(III) or Cu(II) is at rates approaching the diffusion-controlled limit. Thus for ArNO}_2^+ = \text{misonidazole or 5-chloro-1-methyl-4-nitroimidazole:} \\
\text{ArNO}_2^+ + \text{Fe(CN)}_6^{3-} \rightarrow \text{ArNO}_2 + \text{Fe(CN)}_6^{4-} \quad (29)
\]

has $k_{29} = 2.3 \times 10^8$ and $3.6 \times 10^8$ dm$^3$/mole-sec, respectively at zero ionic strength and room temperature (24,81) (both values would be ca. $1 \times 10^9$ dm$^3$/mole-sec in isotonic saline). Electron transfer from ArNO$_2^+$ to Fe(III)-cytochrome c has rate constants of the same order (89).

Reaction of ArNO$_2^+$ with Cu(II) is interesting because of the importance of this center in superoxide dismutase:

\[
\text{ArNO}_2^+ + \text{Cu(II)} \rightarrow \text{ArNO}_2 + \text{Cu(I)} \quad (30)
\]

With 4-nitroacetophenone and Cu(H$_2$O)$_{6}^{2+}$, $k_{30} = 1.4 \times 10^9$ dm$^3$/mole-sec at low ionic strength, decreasing by an order of magnitude or more when Cu(II) is complexed to glycine, tryptophan, etc. (67). A relatively slow reaction of the misonidazole radical-anion with Cu(II) superoxide dismutase was recorded ($k_{30} = 7 \times 10^6$ dm$^3$/mole-sec), but the Cu(II) was essentially inactivated in the reaction, i.e., the type of oxidative, restoration reaction essential to the catalytic reaction with O$_2^{-}$ was not, apparently, favored with ArNO$_2^+$ (67):

\[
\text{ArNO}_2^+ + \text{Cu(I)} \rightarrow \text{ArNO}_2 + \text{Cu(II)} \quad (31)
\]

These studies nonetheless indicated that superoxide dismutase was not inert to electron donors such as some
semiquinones or nitro radical-anions.

The kinetics of electron transfer reactions between ArNO₂⁻/ArNO₂⁺ and other oxidant/radical couples of not too dissimilar reduction potential, e.g., quinones (56,57,80) follow the well-established Marcus-type relationships between the rate constant and free energy (89). Conceptually the simplest electron-transfer reaction is electron exchange between radical-anions and ground states of different (or indeed the same) nitro species:

\[(\text{ArNO}_2^-)_a + (\text{ArNO}_2^-)_b \rightleftharpoons (\text{ArNO}_2^-)_a + (\text{ArNO}_2^+)_b \]  

(32)

Measurements by Dr. I. Wilson (personal communication) for \(\Delta E = -0.36\) to 0.2 V yield:

\[
\log(k_{32}/\text{dm}^3/\text{mole} - \text{sec}) = 11 - 4.90 (1 - 0.86\Delta E)^2
\]

(33)

where

\[
\Delta E = E(\text{ArNO}_2^-/\text{ArNO}_2^+)_b - E(\text{ArNO}_2^-/\text{ArNO}_2^+)_a
\]

(34)

When \(\Delta E = 0\), \(k_{32} = 1.3 \times 10^6 \text{ dm}^3/\text{mole-sec}\), a value around 40-fold slower than the corresponding zero-energy electron-exchange rate with quinones/semi-quinones (89) in aqueous solution.

Reaction (32) is the basis for the facile, chain cis/trans isomerization of the (5-nitro-2-furyl)acrylamide, AF-2, known to occur via nitro radical anion intermediates (11,13,63,69–71). The chain reaction arises because the reduction potential of the \(\text{trans/}	ext{trans}^{\bullet}\) couple is \(\sim 34\) mV lower than that of the \(\text{cis/cis}^{\bullet}\) couple; the chain is propagated via:

\[
\text{trans}^{\bullet} + \text{cis} \rightarrow \text{trans} + \text{cis}^{\bullet}
\]

(35)

competing efficiently with:

\[
\text{cis}^{\bullet} \rightarrow \text{trans}^{\bullet}
\]

(36)

since \(k_{55} = 2 \times 10^6 \text{ dm}^3/\text{mole-sec}\) and \(k_{56} = 5 - 40/\text{sec}\) (71). When AF-2 is used as an indicator of “nitroreductase” activity with other nitroaromatic compounds also present, competing for the (enzyme) electron donor (90), the electron exchange reaction (35) can occur in competition with Eqs. (36) and (2), and the redox-related competition is a reflection of all of these reactions and not just the relative efficiency of electron donation from the enzyme (71).

All the electron-transfer reactions—(27), (29)–(32), and (35)—are typical of intermolecular reactions, of which both the positions of equilibria and the approximate rate constant can be predicted with reasonable confidence, providing estimates are available of the reduction potentials of the donor and acceptor couples. However, intramolecular electron transfer may well be important in particular, substituted nitroaryl radicals. Thus the radical-anions of nitrobenzyl halides undergo intramolecular electron-transfer to yield nitrobenzyl radicals and halide ions (91):

\[
p - \text{NO}_2(\text{C}_6\text{H}_4)\text{CH}_2\text{Br} \rightarrow p - \text{NO}_2(\text{C}_6\text{H}_4)\text{CH}_2^- + \text{Br}^-
\]

(37)

with \(k_{97} = 1.7 \times 10^6/\text{sec}\). Similar behavior is seen with 5- and 8-(halomethyl)-1-nitronaphthalenes and with (4-nitrobenzyl)tosyl derivatives (92). However, 4-haloni-trobenzene radical-anions decay via second-order kinetics (with \(k\) for halide elimination \(< 1/\text{sec}\) (93), and the radical-ion of 4-chloro-1-methyl-5-nitromidazole is similarly long-lived (81). In spite of the apparently normal disproportionation behavior of the latter radical, significant yields of halide ion are eventually produced (at times \(> 1\) sec) following one-electron reduction of this ring-halogenated nitromidazole (81) and analogs (94).
most completely. Using the steady-state approximation: rate of production of $\text{ArNO}_2^+$ = rate of decay, it is easily calculated that the steady-state concentration of nitro radical-anion is ca. 11 nmole/dm$^3$ under these conditions. Then, from Eq. (38), a ratio: rate of restitution/rate of decay = 2000 is expected.

In the same study, it was found that $O_2$ inhibited nitroreduction of four typical 2-nitroimidazoles very much less efficiently than the effect on the reduction of metronidazole or 4-nitroacetophenone. Thus ca. 2–7 µmole/dm$^3$ $O_2$ was required to inhibit effectively the reduction of the 2-nitroimidazoles. The authors pointed out (93) that the differences could arise if there was a first-order pathway for “natural” decay, as indeed had been observed experimentally (21). If the competing reactions are those of Eqs. (2) and (27) with (e.g., metronidazole) $k_d = 4.2 \times 10^6$ dm$^3$/mole-sec and $k_2 = 10^6$ sec$^{-1}$, then an $O_2$ concentration of ca. 20 µmole/dm$^3$ would be required for the rate of Eq. (2) to be ten times faster than the rate of Eq. (27).

Some Other Reactions of Nitro Radicals

The pH-dependent decay of $\text{ArNO}_2^+$ via reactions (21) and (22) is exactly analogous to the decay of $O_2^-$ in aqueous solutions (82), and it is instructive to consider other possible reactions of $\text{ArNO}_2^+$ within the framework of the known chemistry of $O_2^-$. One-electron reduction of several classes of oxidant yields radicals which are more powerful oxidants than the ground state at physiological pH. Examples include: quinones, flavins, and oxygen itself. Alternative statements of this generalization are: $E(A/A^-) - E(A^-/A^2-)$, where A is the oxidant, or the semiquinone formation constant, $K_f = [A^-]/([A][A^{2-}])] < 1$. Thus $O_2^-$ is a more powerful oxidant than $O_2$, however, as discussed above, $\text{ArNO}_2^+$ seems to be a much weaker oxidant than $O_2^-$, since a typical example cannot reoxidize Cu(I).

The reaction of $O_2^-$ with thiols, RSH has been the subject of several studies (96, 97), with the more direct measurements (97) being (in the author's view) the most reliable, especially since the reaction is probably thermodynamically unfavorable (see below). Thus:

$$\text{L-Cysteine} + O_2^- \rightarrow \text{products} \quad (40)$$

was estimated to have $k_{40} < 15 \pm 2$ dm$^3$/mole-sec at pH 10.9 (97). One would expect the reduction by thiolate anions of the weaker oxidant, $\text{ArNO}_2^+$ to be much slower than this latter value. There is some evidence (50, 98) that the reduction potential, $E(\text{RS}/\text{RS}^-)$ does not differ by more than 0.10–0.15 V for the common thiols, so a study with one thiol should be reasonably predictive of the behavior of another.

In spite of this background, and in spite of the total lack of experimental demonstration, several authors have postulated that the protective role of thiols in, e.g., the cytotoxicity of nitroaryl compounds, arises from reaction of $\text{ArNO}_2^+$ with RSH/RS$^-$, presumably the reaction:

$$\text{ArNO}_2^+ + \text{RS}^- + 2 \text{H}^+ \rightarrow \text{ArNO} + \text{RS}^- + \text{H}_2\text{O} \quad (41)$$

This reaction seems exceedingly unlikely thermodynamically. Since thyl radicals, RS oxidize phenothiazines with $k > 3 \times 10^5$ dm$^3$/mole-sec (77), $E(\text{RS}/\text{RS}^-)$ is probably > 1.1 V at pH 3 and at least 0.9 V at pH 7, (probably significantly higher). Since $E(\text{O}_2^-/\text{O}_2^-) = 0.865$ V at pH 7 (58), reaction (40) is probably thermodynamically unfavorable. The couple $\text{ArNO}_2^+/\text{ArNO}$ will only be reversible at high pH, but the difficulty in oxidizing Cu(I) points to its potential being at least several tenths of a volt lower than that of RS/RS$^-$. Reaction (41) thus seems most unfavorable, unless irreversibility facilitates the forward reaction. This analysis is entirely consistent with two independent, experimental studies. Polnaszek et al. (99) found that 0.1 mole/dm$^3$ glutathione (GSH) had no effect on the steady-state concentration of $\text{ArNO}_2^+$ produced via rat hepatic microsomal or xanthine oxidase reducing systems, using three different 5-nitrofurans. The lifetime of misonidazole or metronidazole radical-anions was not detectably changed by the presence of GSH (2 mmole/dm$^3$) at pH 7.3 or 9.4 (the higher pH should favor reaction (41)); thus $k_{41} < 5$ dm$^3$/mole-sec at pH 9.4 (21) and is very probably < 0.05 dm$^3$/mole-sec at pH 7.4.

The well-characterized reaction of nitrosoaryl compounds with GSH (100) is, therefore, probably > 6 orders of magnitude faster than Eq. (41) and the most likely explanation of the biological role of GSH in nitroaryl cytotoxicity, etc. However, nitroaryl compounds, when coreduced with DNA, do cause extensive damage to the macromolecule (101) in spite of a lack of effect of DNA, RNA, ribose, nucleotides or protein on the steady-state concentration of $\text{ArNO}_2^+$ in reductase systems (99). We have failed to demonstrate any oxidizing properties of $\text{ArNO}_2^+$ with some of the most favorable possible reductants, e.g., N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) (80):

$$\text{ArNO}_2^+ + \text{TMPD} + 2 \text{H}^+ \rightarrow \text{ArNO} + \text{TMPD}^+ + \text{H}_2\text{O} \quad (42)$$

Oxidation of e.g., guanine sites, G would be much less favorable than Eq. (42) since $E(\text{G}^+/\text{G}) >> E(\text{TMPD}^+/\text{TMPD})$. We have speculated that nitroso radicals could be more powerful oxidants than $\text{ArNO}_2^+$ (50), since nitrosobenzene is a more powerful oxidant than nitrobenzene (18):

$$\text{ArNO}_2^+ + \text{ArNO} \rightarrow \text{ArNO}_2 + \text{ArNO}^+ \quad (43)$$

and indeed than oxygen (30):

$$O_2^+ + \text{ArNO} \rightarrow \text{O}_2 + \text{ArNO}^+ \quad (44)$$

with $k_{43} = 4.1 \times 10^7$ dm$^3$/mole-sec (18) and $k_{44} = 4 \times 10^6$ dm$^3$/mole-sec (E. D. Clarke, personal communication).
tion).

ArNO₂⁺ is, of course, protonated at pH 7 and this recalls a note of caution (50) concerning the potential reactivity of ArNO₂⁻. Since HO₂ is several orders of magnitude more reactive than O₂⁻ in some circumstances (10²), it is possible that the protonated conjugate of ArNO₂⁻ may play a role in its biological activity.

Conclusions

Although a detailed understanding of the mechanisms of cytotoxicity of nitroaryl compounds in both procarcyotic and eucaryotic cells still eludes us, there is no question that the use of these compounds in medicine and cancer therapy relies upon free-radical mechanisms. The redox properties of the one-electron couple: ArNO₂/ArNO₂⁻ define virtually all the biological properties of these compounds. Disproportionation of the radicals controls their natural lifetime in most model chemical and biological systems (though with important exceptions). In all cases, the rates of these natural radical-decay processes is a function of pH and the prototropic properties of the radical. Most simple electron-transfer reactions can be rationalized in terms of both equilibrium and kinetics.

However, there are still many, important questions unanswered. There are some parallels in the chemistry of ArNO₂⁻ and O₂⁻. The enormous, widespread interest in the biological role of O₂⁻ is somewhat paradoxical since O₂⁻ itself is really rather an unreactive species. It seems that ArNO₂⁻ is even less reactive than O₂⁻ towards likely biological targets (except of course readily definable electron acceptors). It is hoped that this short article will help clarify the likely role of nitro radicals in biological systems and help point experimentalists towards identifying the critical reactions, which may well involve ArNO₂⁻ as an obligate intermediate but probably not as the direct, damaging toxin.

This work was supported by the Cancer Research Campaign.

REFERENCES

1. Docampo, R., Moreno, S. N. J., Stoppani, A. O. M., Leon, W., Cruz, F. S., Villalta, F., and Muniz, R. P. A. Mechanism of nitrosim toxicitiy in different forms of Trypanosoma cruzi. Biochem. Pharmacol. 30: 1947–1951 (1981).

2. Moreno, S. N. J., Mason, R. P., Muniz, R. P. A., Cruz, F. S., and Docampo, R. Communication: generation of free radicals from metronidazole and other nitroimidazoles by Trichomonas foetus. J. Biol. Chem. 265: 4081–4084 (1990).

3. Moreno, S. N. J., Mason, R. P., and Docampo, R. Distinct reduction of nitrofurans and metronidazole to free radical metabolites by Trichomonas foetus hydrogenosomal and cytosolic enzymes. J. Biol. Chem. 259: 8253–8259 (1984).

4. Wardman, P., and Clarke, E. D. Oxygen inhibition of nitroreductase: electron transfer from nitro radicals to oxygen. Biochem. Biophys. Res. Commun. 69: 942–949 (1976).

5. Wardman, P. Application of pulse radiolysis methods to study the reactions and structure of biomolecules. Repts. Prog. Phys. 41: 259–302 (1978).

6. Mason, R. P., and Holtzman, J. L. ESR spectra of free radicals formed from nitroaromatic drugs by microsomal nitroreductase. Pharmacologist 16: 277 (1974).

7. Mason, R. P., and Holtzman, J. L. The mechanism of microsomal and mitochondrial nitroreductase. Electron spin resonance evidence for nitroaromatic free radical intermediates. Biochemistry 14: 1636–1632 (1975).

8. Mason, R. P., and Holtzman, J. L. The role of catalytic superoxide formation in the O₂ inhibition of nitroreductase. Biochem. Biophys. Res. Commun. 67: 1267–1274 (1975).

9. Mason, R. P. Free radical metabolites of foreign compounds and their toxicological significance. In: Reviews in Biochemical Toxicology, Vol. 1 (E. Hodgson, J. R. Bend, and R. M. Philip, Eds.) Elsevier North Holland, New York, 1979, pp. 151–200.

10. Peterson, F. J., Mason, R. P., Hovsepian, J., and Holtzman, J. L. Oxygen-sensitive and -insensitive nitroreduction by Escherichia coli and rat hepatic microsomes. J. Biol. Chem. 254: 4009–4014 (1979).

11. Kalyanaraman, B., Perez-Reyes, E., Mason, R. P., Peterson, F. J., and Holtzman, J. L. Electron spin resonance evidence for a free radical intermediate in the cis-trans isomerization of furyluramides by oxygen-sensitive nitroreductases. Mol. Pharmacol. 16: 1059–1064 (1979).

12. Perez-Reyes, E., Kalyanaraman, B., and Mason, R. P. The reductive metabolism of metronidazole and ronidazole by aerobic liver microsomes. Mol. Pharmacol. 17: 239–244 (1980).

13. Kalyanaraman, B., Mason, R. P., Rowlett, R., and Kispert, L. D. An electron spin resonance investigation and molecular orbital calculation of the anion radical intermediate in the enzyme-catalysed cis-trans isomerization of furyluramides, a nitrofuran derivative of ethylene. Biochem. Biophys. Acta 660: 102–109 (1981).

14. Docampo, R., Mason, R. P., Mottley, C., and Munis, R. P. A. Generation of free radicals induced by nifurtimox in mammalian tissues. J. Biol. Chem. 256: 10930–10933 (1981).

15. Moreno, S. N. J., Docampo, R., Mason, R. P., Leon, W., and Stoppani, A. O. M. Different behaviours of benznidazole as free radical generator with mammalian and Trypanosoma cruzi microsomal preparations. Arch. Biochem. Biophys. 218: 585–591 (1982).

16. Mason, R. P. Free-radical intermediates in the metabolism of toxic chemicals. In: Free Radicals in Biology, Vol. V, (W. A. Pryor, Ed.), Academic Press, New York, 1982, pp. 161–222.

17. Sealy, R. C., Swartz, H. M., and Olive, P. L. Electron spin resonance-spin trapping. Detection of superoxide formation during aerobic microsomal reduction of nitro-compounds. Biochim. Biophys. Res. Commun. 82: 680–684 (1978).

18. Asmus, K.-D., Wigger, A., and Henglein, A. Pulsradiolytische Untersuchung einiger Elementarprozesse der Nitrobenzolreduktion. Ber. Bunsenges. Phys. Chem. 70: 862–868 (1966).

19. Corvaja, C., Farnia, G., and Vianello, E. Kinetics of decay of nitrophenol radical anions and reduction mechanism of nitrophenols in aqueous alkaline media. Electrochim. Acta 11: 919–929 (1965).

20. Neta, P., Simic, M. G., and Hoffman, M. Z. Pulse radiolysis and electron spin resonance studies of nitroaromatic radical anions. Optical absorption spectra, kinetics, and one-electron redox potentials. J. Phys. Chem. 80: 2018–2023 (1976).

21. Wardman, P. Lifetimes of the radical anions of medically-important nitroaryl compounds in aqueous solution. Life Chem. Repts. 3: 22–28 (1987).

22. Grivel, M., Feijik, A., and Henglein, A. Pulsradiolytische Bestimmung der Absorptions-spektren und Dissoziationskonstanten kurzlebiger halborduzierer aromatischer Nitroverbindungen. Z. Naturforsch. 24b: 1386–1388 (1969).

23. Greenstock, C. L., Dunlop, I., and Neta, P. Radiation chemical studies of the oxidation and reduction of nitrofurans. Oxidative denitrification by OH radicals. J. Phys. Chem. 77: 1197–1190 (1973).

24. Wardman, P. Protonation of the radical-anions of nitro-imidazolone radiosensitizers and the formation of radical-adducts. Int. J. Radiat. Biol. 28: 585–588 (1975).

25. Biaglow, J. E., and Durand, R. E. The effects of nitrobenzene derivatives on oxygen utilization and radiation response of an in vitro tumor model. Radiat. Res. 65: 529–539 (1976).

26. Durand, R. E., Biaglow, J. E., and Sutherland, R. M. Hypoxic radiosensitizers and cellular respiration. Brit. J. Radiol. 49: 567–
568 (1976).
27. Biaglow, J. E. Cellular electron transfer and radical mechanisms for drug metabolism. Radiat. Res. 86: 212–242 (1981).
28. Durand, R. E., and Olive, P. L. Evaluation of nitrohydrocyclic radiosensitizers using spheroids. Adv. Radiat. Biol. 9: 75–107 (1981).
29. Biaglow, J. E., Varnes, M. E., Koch, C. J., and Shridhar, R. The metabolic activation of carcinogenic nitro compounds to oxygen-reactive intermediates. In: Free Radicals and Cancer (R. A. Floyd, Ed.), Marcel Dekker, New York, 1982, pp. 441–502.
30. Wardman, P. The use of nitroaromatic compounds as hypoxic cell radiosensitizers. Curr. Topics Radiat. Res. Q. 11: 347–398 (1977).
31. Adams, G. E., Fowler, J. F., and Wardman, P. (Eds.) Hypoxic Cell Sensitizers in Radiobiology and Radiotherapy. Brit. J. Cancer (Suppl.) 37: III (1976).
32. Brady, L. W. (Ed.) Radiation Sensitizers. Their Use in the Clinical Management of Cancer. Masson, New York, 1980.
33. Sutherland, R. M. (Ed.) Conference on chemical modification: radiation and cytotoxic drugs. Int. J. Radiat. Oncol. Biol. Phys. 8: 323–815 (1982).
34. Chapman, J. D., and Whitmore, G. F. (Eds.) Chemical modifiers of cancer treatment. Int. J. Radiat. Oncol. Biol. Phys. 10: 1161–1813 (1984).
35. Bors, W., Saran, M., and Tait, D. (Eds.) Oxygen Radicals in Chemistry and Biology. Walter de Gruyter, Berlin, 1984.
36. Halliwell, B., and Gutteridge, J. M. C. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem. J. 219: 1–14 (1984).
37. Borg, D. C., and Schai, K. M. Cytotoxicity from coupled redox cycling of autooxidizing xenobiotics and metals. A selective critical review and commentary on work-in-progress. Isr. J. Chem. 24: 58–53 (1984).
38. Sutherland, R. M. Selective chemotherapy of noncycling cells in an in vitro tumor model. Cancer Res. 34: 3501–3508 (1974).
39. Hall, E. J., and Roizin-Towle, L. Hypoxic sensitizers: Radiobiological studies at the cellular level. Radiology 117: 453–457 (1975).
40. Modhindra, J. K., and Rauth, A. M. Increased cell killing by metronidazole and nitrofurazone of hypoxic compared to aerobic mammalian cells. Cancer Res. 36: 950–968 (1976).
41. Moreno and Docampo, Environ. Health Perspect. 64: 199–208 (1985).
42. Joseph, P. D., and Mason, R. P. Chemical and enzymatic nitro-reduction: free radical and diamagnetic products of nitroimidazoles. In: Bioactivation of Foreign Compounds (M. W. Anders, Ed.), Academic Press, New York, 1985.
43. Raleigh, J. A., and Liu, S. F. Reductive fragmentation of 2-nitroimidazoles in the presence of nitroreductases—glyoxal formation from misonidazole. Biochem. Pharmacol. 32: 1444–1446 (1983).
44. McCalland, R. A., Fuller, J. R., Seaman, N. E., Rauth, A. M., and Battistella, R. 2-Hydroxylaminimidazoles - unstable intermediates in the reduction of 2-nitroimidazoles. Biochem. Pharmacol. 33: 303–309 (1984).
45. Varghese, A. J. Glutathione conjugates of misonidazole. Biochem. Biophys. Res. Commun. 112: 1013–1020 (1983).
46. Varghese, A. J., and Whitmore, G. F. Detection of a reactive metabolite of misonidazole in hypoxic mammalian cells. Radiat. Res. 97: 262–271 (1984).
47. Smith, B. R. Hypoxia-enhanced reduction and covalent binding of [2-H] misonidazole in the perfused rat liver. Biochem. Pharmacol. 33: 1579–1581 (1984).
48. Masana, M., de Toranzo, E. G. D., and Castro, J. A. Reductive metabolism and activation of benzimidazole. Biochem. Pharmacol. 33: 1041–1045 (1984).
49. Watta, M. E., Anderson, R. F., Jacobs, R. S., Patel, K. B., Wardman, P., Woodcock, M., Smithen, C. E. Moazzam, M., Parrick, J., and Wallace, R. G. Evaluation of novel hypoxic cell radiosensitizers in vitro. The value of studies in single-cell systems. In: Radiation Sensitizers: Their Use in the Clinical Management of Cancer (L. W. Brady, Ed.), Masson, New York, 1980, pp. 175–185.
50. Wardman, P. Electron transfer and radical-addition in the radiosensitization and chemotherapy of hypoxic cells. Proceedings 4th International Symposium, Hypoxic Cell Radiosensitizing Drugs: The First and Second Generation Compounds for Cancer Treatment (A. Brecia, Ed.), 1985, in press.
51. Reynolds, A. V. The activity of nitro-compounds against Bacteriodes fragilis is related to their electron affinity. J. Antiimicrob. Chemotherapy. 8: 91–99 (1981).
52. Verplanken, H. A., and De Ranter, C. J. Relationships between physicochemical parameters and antimicrobial activity of a series of 5-nitroimidazoles. In: Quantitative Approaches to Drug Design (J. D. Dearden, Ed.), Elsevier, Amsterdam, 1983, pp. 280–281.
53. Yarlett, N., Gorrell, T. E., Marczak, R., and Müller, M. Reduction of nitroimidazole derivatives by hydrogenosomal extracts of Trichomonas vaginalis. Mol. Biochem. Parasitol. 14: 29–40 (1985).
54. Brecia, A., Berrilli, G., and Roffia, S. Chemical radiosensitization of hypoxic cells and redox potentials: Correlation of volumetric results with pulse radiolysis data of nitrocompounds and radiosensitizers. Int. J. Radiat. Biol. 36: 85–99 (1979).
55. Olive, P. L. Correlation between metabolic reduction rates and electron affinity of nitroheterocycles. Cancer Res. 39: 4512–4515 (1979).
56. Meisel, D., and Neta, P. One-electron redox potentials of nitro compounds and radiosensitizers. Correlation with spin densities of their radical anions. J. Am. Chem. Soc. 97: 5188–5206 (1976).
57. Wardman, P., and C. D. One electron reduction potentials of substituted nitroimidazoles measured by pulse radiolysis. J. Chem. Soc. Faraday Trans. I. 72: 1377–1390 (1976).
58. Meisel, D., and Czapski, G. One-electron transfer equilibria and redox potentials of radicals studied by pulse radiolysis. J. Phys. Chem. 79: 1505–1509 (1975).
59. Wardman, P. Molecular structure and biological activity of hypoxic cell radiosensitizers and hypoxia-specific cytotoxins. In: Advanced Topics on Radiosensitizers of Hypoxic Cells (A. Brecia, C. Rimondi, and G. E. Adams, Eds.) Plenum Press, New York, 1982, pp. 49–75.
60. Biaglow, J. E., Jackson, B., and Koch, C. The catalytic effect of the carcinogen “4-nitroquinoline-N-oxide” on the oxidation of vitamin C. Biochem. Biophys. Res. Comm. 70: 1316–1323 (1976).
61. Biaglow, J. E., Jackson, B., Varnes, M., and Koch, C. The oxidation of ascorbate by electron affiine drugs and carcinogens. Photochem. Photobiol. 28: 869–876 (1978).
62. Bielski, B. H. J. Chemistry of ascorbic acid radicals. In: Ascorbic Acid Chemistry, Metabolism and Uses (P. A. Seib and B. M. Tolbert, Eds.) Adv. Chem. Ser. 200, American Chemical Society, Washington, DC, 1982, pp. 81–100.
63. Clarke, E. D. Wardman, P., and Wilson, I. Cis-trans isomerization of the (5-nitro-2-furyl)acrylamide, AF-2, initiated by ascorbate, glutathione, Fe(II) and OH•. Biochem. Pharmacol. 33: 37–87 (1984).
64. Steeneken, S., and Neta, P. Electron transfer rates and equilibria between substituted phenoxime ions and phenoxyl radicals. J. Phys. Chem. 83: 1134–1137 (1979).
65. Wardman, P. Some applications of radiation chemistry to biochemistry and radiobiology. In: Radiation Chemistry: Principles and Applications (M. A. J. Rodgers and Farhataziz, Eds.), VCH Publishers, 1985, in press.
66. Weis, W. Ascorbic acid and electron transport. Ann. N. Y. Acad. Sci., 258: 190–200 (1975).
67. Wardman, P. Specificity of superoxide dismutase in catalysing redox reactions: A pulse radiolysis study. In: Radiation Biology and Chemistry: Research Development (H. E. Edwards, S. Navaratnam, B. J. Parsons, and G. O. Phillips, Eds.), Elsevier, Amsterdam, 1979, pp. 189–196.
68. Cabelli, and Bielski, B. H. J. Kinetics and mechanism for the oxidation of ascorbic acid/ascorbate by HO2/ O•- radicals. A pulse radiolysis and stopped-flow photolysis study. J. Phys. Chem. 87: 1809–1812 (1983).
69. Tatsunami, K., Kitamura, S., Koga, N., Yoshimura, H., and Kato, Y. Cis-trans isomerization of nitrofurane derivatives by xanthine oxidase. Biochem. Biophys. Res. Commun. 73: 947–952 (1978).
70. Tatsumi, K., Koga, N., Kitamura, S., Yoshimura, H., Wardman, P., and Kato, Y. Enzymic cis-trans isomerization of nitrofuran derivatives. Isomerizing activity of xanthine oxidase, lipoyl dehydrogenase, DT-diaphorase and liver microsomes. Biochim. Biophys. Acta 567: 75–87 (1979).

71. Clarke, E. D. Wardman, P., and Wilson, I. The mechanism of the free-radical-induced chain isomerisation of 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide. J. Chem. Soc. Perkin Trans. II: 1155–1161 (1984).

72. Anderson, R. F. Energetics of the one-electron reduction steps of riboflavin, FMN and FAD to their fully reduced forms. Biochem. Biophys. Acta. 722: 158–162 (1983).

73. Clarke, E. D., Wardman, P., and Goulding, K. H. Anaerobic reduction of nitroimidazoles by reduced flavin mononucleotide and by xanthine oxidase. Biochem. Pharmacol. 29: 2894–2897 (1980).

74. Willson, R. L., and Searle, A. J. F. Metronidazole (Flagyl): ion catalysed reaction with sulphhydryl groups and tumour radiosensitization. Nature, 255: 498–500 (1975).

75. Bahmann, D., Basaga, H., Dunlop, J. R., Searle, A. J. F., and Willson, R. L. Metronidazole (Flagyl), misonidazole (Ro 07-0582), iron, zinc and sulphur compounds in cancer therapy. Brit. J. Cancer (Suppl. III) 37: 16–19 (1978).

76. Yano, Y., Sakaguchi, T., and Nakazato, M. Marked metal ion effects in electron transfer from reduced flavin to aromatic nitro compounds in ethanol. J. Chem. Soc. Perkin Trans. II: 585–599 (1984).

77. Forni, L. G., Mönig, J., Mora-Arellano, V. O., and Willson, R. L. Thiyl free radicals: direct observations of electron transfer reactions with phenothiazines and ascorbate. J. Chem. Soc. Perkin Trans. II: 961–965 (1983).

78. Adams, G. E., and Willson, R. L. Ketyl radicals in aqueous solution. Pulse radiolysis study. J. Chem. Soc. Faraday Trans. I 69: 719–729 (1973).

79. Sjöberg, L., and Eriksen, T. E. Nitrobenzenes: a comparison of pulse radiolytically determined one-electron reduction potentials and calculated electron affinities. J. Chem. Soc. Faraday Trans. I 76: 1402–1408 (1980).

80. Greenstock, C. L., and Ruddock, G. W. Pulse radiolysis and ESR studies of the electron-affinic properties of nitroheterocyclic radiosensitizers. Radiat. Res. 66: 472–484 (1976).

81. Clarke, E. D., and Wardman, P. Are ortho-substituted 4-nitroimidazoles a new generation of radiation-induced arylating agents? Int. J. Radiat. Biol. 37: 463–468 (1980).

82. Ayers, P. B., Elliott, A. J., and Salmon, G. A. In situ radiolysis electron spin resonance study of the radical-anions of substituted nitroimidazoles and nitroaromatic compounds. J. Chem. Soc. Faraday Trans. I 74: 511–518 (1978).

83. Bielski, B. H. J. Reevaluation of the spectral and kinetic properties of HO2 and O2- free radicals. Photochem. Photobiol. 28: 645–649 (1978).

84. Mason, R. P., and Holtzman, J. L. The kinetics of nitroreductase anion radical intermediates. Fed. Proc. 34: 865 (1975).

85. Kastening, B. Elektrochemische Bildung, Reactivität und Eigenschaften des Nitrobenzol Radicalansions. Electrochem. Acta 9: 241–254 (1964).

86. von Koopmann, R., and Gerischer, H. Untersuchung der elek trochemischen Reduktion von Nitrobenzol durch Kombination von ESR-Messungen mit elektroanalytischen Methoden. Ber. Bunsenges. Phys. Chem. 70: 127–138 (1966).

87. Greenstock, C. L., Dunlop, J. Pulse radiolysis studies of nitrofurans: chemical radiosensitization. Radiat. Res. 56: 428–440 (1973).

88. Greenstock, C. L., Biaglow, J. E., and Durand, R. E. Effects of sensitizers on cell respiration: II. The effects of hypoxic cell sensitizers on oxygen utilization in cellular and chemical models. Br. J. Cancer (Suppl. III) 37: 11–15 (1978).

89. Meisel, D. Free energy correlation of rate constants for electron transfer between organic systems in aqueous solutions. Chem. Phys. Letters 34: 263–266 (1975).

90. Raleigh, J. A., Shum, F. Y., and Liu, S. F. Nitroreductase-induced binding of nitroaromatic radiosensitizers to unsaturated lipids. Biochem. Pharmacol. 30: 2921–2925 (1981).

91. Neta, P., and Behar, D. Intramolecular electron transfer in the anion radicals of nitrobenzyl halides. J. Am. Chem. Soc. 102: 4798–4802 (1980).

92. Bays, J. P., Blumer, S. T., Barai-Tosh, S., Behar, D., and Neta, P. Intramolecular electron transfer and dehalogenation of nitroaromatic anion radicals. J. Am. Chem. Soc. 105: 320–324 (1983).

93. Behar, D., and Neta, P. Intramolecular electron transfer and dehalogenation of anion radicals. 2. Halonitroaromatic compounds. J. Phys. Chem. 85: 690–693 (1981).

94. Ma, H., Hardy, C. R., and O’Neill, P. Formation of halide-ions on one-electron reduction of halogenated nitroimidazoles in aqueous solution. A radiolytic study. Int. J. Radiat. Biol. 41: 151–160 (1982).

95. Rauth, A. M., McClelland, R. A., Michaels, H. B., and Battista, R. The oxygen dependence of the reduction of nitroimidazoles in a radiolytic model system. Int. J. Radiat. Oncol. Biol. Phys. 10: 1323–1326 (1984).

96. Asada, K., and Kamematsu, S. Reactivity of thiol with superoxide radicals. Agr. Biol. Chem. 40: 1891–1892 (1976).

97. Bielski, B. H. J., and Shue, G. G. Reaction rates of superoxide radicals with the essential amino acids. In: Oxygen Free Radicals and Tissue Damage, Ciba Foundation Symp. 65 (new series), Excerpta Medica, Amsterdam, 1979, pp. 45–48.

98. Stricks, W., Frischmann, J. K., and Mueller, R. G. Polarography of mercaptalkyl compounds and their disulphides. J. Electrochem. Soc. 109: 518–521 (1962).

99. Polanszek, C. F., Peterson, F. J., Holtzman, J. L., and Mason, R. P. No detectable reaction of the anion radical metabolite of nitrofurans with reduced glutathione or macromolecules. Chem.- Bioi. Interact. 51: 263–271 (1984).

100. Diepold, C., Eyer, P., Rappmayer, H., and Reinhardt, K. Reactions of aromatic nitroso compounds with thiols. In: Biological Reactive Intermediates - II. Chemical Mechanisms and Biological Effects. (R. Snyder, D. J. Jollow, D. V. Parke, C. G. Gibson, J. J. Koescia, C. M. Wittmer, Eds.), Plenum Press, New York, 1982, pp. 1179–1181.

101. Edwards, D. I. Mechanisms of cytotoxicity of nitroimidazole drugs. Progr. Med. Chem. 18: 88–116 (1981).

102. Gebicki, J. M., and Bielski, B. H. Comparison of the capacities of the perhydroxyl and the superoxide radicals to initiate chain oxidation of linoleic acid. J. Am. Chem. Soc. 103: 7020–7022 (1981).