Redox Cycling of Radical Anion Metabolites of Toxic Chemicals and Drugs and the Marcus Theory of Electron Transfer

by Ronald P. Mason*

A wide variety of aromatic compounds are enzymatically reduced to form anion free radicals that generally contain one more electron than their parent compounds. In general, the electron donor is any of a wide variety of flavoenzymes. Once formed, these anion free radicals reduce molecular oxygen to superoxide and regenerate the parent compound unchanged. The net reaction is the oxidation of the flavoenzyme's coenzymes and the reduction of molecular oxygen. This catalytic behavior has been described as futile metabolism or redox cycling. Electron transfer theory is being applied to these reactions and, in some cases, has successfully correlated $V_{max}$ and $K_m$ with the reduction potentials of the aromatic compounds.

Introduction

A free radical is any organic molecule with an odd number of electrons. Even a simple organic molecule such as benzene can be transformed into three chemically distinct, highly reactive free radicals (Fig. 1). One-electron oxidation, the removal of an electron from the pi-electrons, results in the formation of the benzene cation radical. The one-electron reduction of benzene, the addition of an electron, results in the formation of the benzene anion radical. The third free radical is formed by the homolytic cleavage of one of the C-H bonds by UV light or other radiation to form a hydrogen atom and the phenyl radical.

Severe chemical conditions are necessary to form free radicals from benzene, but this is not the case for most aromatic compounds. In fact, many classes of free radicals are formed as a result of the metabolism of chemicals. In our work, we delineate the metabolic pathways by which a given class of free radicals may be formed, the subsequent reactions of these free radicals under physiological conditions, and the toxicological implications of these reactions. Of the three types of free-radical metabolites, only radical anion metabolites participate in redox cycling. These species are analogous to the benzene anion (Fig. 1). They are formed by a one-electron transfer from an enzyme to an aromatic organic chemical, which may be either a drug or an industrial chemical. Investigations of bipyridylium, azo, quinone and nitro radical anion metabolites have been extensively studied (1-4).

Paraquat and Other Bipyridylium Compounds

The herbicide paraquat and related bipyridylium dications such as diquat can undergo a one-electron reduction to form very stable free radicals. In 1933, Michaelis and Hill (5) showed that the paraquat free radical can use molecular oxygen as a one-electron acceptor to form the superoxide anion radical with the regeneration of the paraquat dication (5) [Eq. (1)].

$$\text{PQ}^{++} + \text{O}_2 \rightarrow \text{PQ}^{2+} + \cdot\text{O}_2^-$$

(1)

In 1960, Homer and others (6) proposed that the reduction of paraquat to its free radical was an essential step in its herbicidal mode of action, because a correlation was found between the reduction potential of paraquat analogs and their herbicidal activity (6). Paraquat is reduced to its free radical within chloroplasts during photosynthesis, and the herbicidal activity of
parquat requires light for electron transport. Plant leaves incubated in parquat solutions accumulated malondialdehyde, indicating that lipid peroxidation occurs (7). This lipid peroxidation is thought to be mediated by the one-electron reduction of parquat and the subsequent transfer of the electron to molecular oxygen resulting in superoxide formation (8).

For 20 years, parquat has been known to be reduced in anaerobic microsomal incubations to a free radical, as evidenced by its visible absorption spectrum (9). This free radical has a deep blue color. The electron spin resonance spectrum (ESR) is a better means of identification of free radicals because, like nuclear magnetic resonance, ESR is much more specific than UV-visible spectroscopy. Paraquat serves as an ideal model compound for investigating free radical-mediated toxicity because it has no known metabolism other than the free-radical metabolism.

In microsomal systems, the enzymatic reduction of parquat to its cation radical is catalyzed by the flavoenzyme NADPH-cytochrome P-450 reductase (Fig. 2). The parquat radical is stable in the absence of oxygen. In the presence of oxygen, parquat is reformed and superoxide is generated in a catalytic fashion with no net change occurring to the parquat molecule (Fig. 2). This process has been termed futile metabolism (1, 3) or redox cycling (4). The mechanism of parquat poisoning in man and other mammals is generally thought to be a superoxide-mediated toxicity that is completely analogous to the herbicidal mode of action. The lung is the site of injury because of the accumulation of parquat in this tissue (10). The energy-dependent uptake of parquat and the subsequent free-radical formation are cell-specific. Paraquat free-radical formation occurs with Clara cells and alveolar Type II cells but not with alveolar macrophages (11). Diquat, morfamquat, and other bipyridylum compounds do not affect the lung as seriously, but these compounds do cause liver damage. We have shown that diquat, paraquat, benzyl viologen, and morfamquat are reduced by rat hepatocytes to their respective radical cations (12).

Quinones

The quinone moiety is found in pigments isolated from a variety of plants and fungi, some of which are clinically important anticancer drugs (13). Although menadione (vitamin K₃) is used therapeutically, it is also cytotoxic and causes the marked decrease of intracellular thiols such as glutathione, the formation of superoxide by futile cycling (Fig. 3), the oxidation of reduced pyridine nucleotides (Fig. 3), alterations in intracellular calcium ion homeostasis, and the death of isolated hepatocytes (14, 15).

Doxorubicin, daunorubicin, and other anthracycline anticancer drugs are known to be carcinogenic, mutagenic, and cardiotoxic (18). The first evidence of enzymatic semiquinone formation from a quinone anticancer drug was indirect. In 1975, Handa and Sato (16) demonstrated that daunorubicin and doxorubicin mediated the formation of superoxide in microsomal incubations containing NADPH (16). Later, they also demonstrated that these compounds stimulated aerobic NADPH oxidation in the absence of any net reduction of these antimetabolites (17). The presence of semiquinone metabolites of anthracyclines has been demonstrated with ESR in anaerobic incubations containing microsomes, purified NADPH-cytochrome P-450 reductase, and even in incubations of tumor cells (9). Analysis of the high-resolution ESR spectrum of the enzymatically generated daunorubicin semiquinone was reported recently (18).

Azo Compounds

Although red dye number 2 (Fig. 4) is only a weak carcinogen, this compound was recently banned as a food dye by the Food and Drug Administration because of its high consumption. The reductive metabolism of azo compounds such as red dye number 2 by a wide variety of biological systems has long been known. Sulfonyl III is a diazophenothol compound that is used in the titrimetric determination of sulfates and organic sulfur (Fig. 5); it is structurally related to the monoazo food dyes such as red dye number 2. We have detected the ESR spectrum of a free-radical metabolite of sulfonyl III in anaerobic rat hepatic microsomal incubations containing this azo dye and NADPH (19). NADPH is the ultimate source of the electron.

The spectrum of the sulfonyl III free radical is characterized by a partially resolved 17-line hyperfine pattern and a g-value of the center line equal to 2.0034 (Fig. 5). The g-value is analogous to the chemical shift in nuclear magnetic resonance and is used to charac-

**Figure 2.** Futile metabolism or redox cycling of parquat by NAD(P)H-dependent flavoenzymes.

**Figure 3.** Futile metabolism or redox cycling of many quinones by NAD(P)H-dependent flavoenzymes.

**Figure 4.** Structure of red dye number 2.
terize the structure of the free radical. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) serves as a g-value standard analogous to tetramethylsilane in NMR. The eight lines upfield and eight lines downfield of the center line indicate that the unpaired electron is delocalized onto at least one of the aromatic rings and probably onto both azo groups. Again, the scheme of futile metabolism emphasizes the rapid air oxidation of the azo anion radical as the pivotal event (20). In such a scheme there would be no net reduction of the azo compound since the parent compound would be reformed (Fig. 6). Sulfonazo III would thereby catalyze the production of superoxide anion radical and oxygen consumption.

The simplest method for detecting superoxide is by the addition of superoxide dismutase to the reaction medium. This enzyme catalyzes the disproportionation of the superoxide anion radical to give back half of the superoxide as oxygen and reduces the other half to hydrogen peroxide [Eq. (2)].

\[ 2^\cdot O_2 + 2H^+ \rightarrow O_2 + H_2O_2 \]  

(2)

In such a reaction, the hydrogen peroxide formed by the disproportionation of the superoxide anion radical can itself be disproportionated by catalase to give back half of the oxygen as molecular oxygen [Eq. (3)].

\[ 2H_2O_2 \rightarrow O_2 + 2H_2O \]  

(3)

When both superoxide dismutase and catalase are added to the incubation, water is the only reduced species of oxygen that can accumulate.

In view of these considerations, the stimulation of oxygen uptake by sulfonazo III and the reversal of this stimulation by superoxide dismutase and catalase would be a useful approach to determine whether the azo anion radical is formed in the presence of oxygen. When we examined the effect of 50 μM sulfonazo III on the NADPH-supported oxygen consumption by rat hepatic microsomes, we found that, indeed, the rate of oxygen uptake was increased 10-fold over the basal rate and that this stimulation was partially reversed by superoxide dismutase (Fig. 7). The presence of the superoxide anion radical strongly suggested that the sulfonazo anion free radical is formed by a microsomal reductase under aerobic conditions. The rate of oxygen uptake is over five times greater than that observed during normal cytochrome P-450-catalyzed reactions. As expected, the disproportionation of hydrogen peroxide by catalase also decreased the sulfonazo III-stimulated uptake of oxygen (Fig. 7). The rate of dye disappearance in these incubations is only 2% of the rate of oxygen consumption. This implies that the consumption of oxygen is indeed catalytic, as is consistent with the scheme (Fig. 6). The oxidation of NADPH by microsomal incubations is also greatly increased by sulfonazo III, but it is not influenced by superoxide dismutase or catalase (20).

One final point is that sulfonazo III anion radical for-

![Figure 5](image-url)  
**FIGURE 5.** The ESR spectrum of the sulfonazo III free radical detected in anaerobic microsomal incubations containing an NADPH-generating system. The g-value of 2,2-diphenyl-1-picrylhydrazyl (DPPH) is indicated by the arrow. From Mason et al. (20).

![Figure 6](image-url)  
**FIGURE 6.** Futile metabolism or redox cycling of some azo compounds by NAD(P)H-dependent flavoenzymes.

![Figure 7](image-url)  
**FIGURE 7.** Effect of superoxide dismutase and catalase on sulfonazo III stimulation of oxygen consumption by rat hepatic microsomal incubations. Data from Mason et al. (20).
Nitroaromatic Compounds

Nitroaryl and nitroheterocyclic compounds have enjoyed widespread use in medicine as antibiotics (Table 1). The most widely employed topical substituted 5-nitrofuran, nitrofurazone, has been used as a food preservative, in therapy of patients with second- and third-degree burns, and as an antibacterial agent for the treatment or prevention of a wide variety of infections of the genito-urinary tract. Nitrofurantoin is the substituted 5-nitrofuran administered most frequently for systemic infections, particularly those involving the urinary tract. Benznidazole has found use as an antiprotozoal (21), and metronidazole has been widely used for many years in the treatment of infections of Trichomonas vaginalis, amoebas and Giardia, and a host of anaerobic bacterial infections.

In our early investigation of the mechanism of rat hepatic microsomal and microsomal nitroreductase (22), we reported ESR and kinetic evidence that suggested that the first step in these nitroreductase reactions is the transfer of a single electron to nitro compounds to give the corresponding nitro anion free radical. For instance, in the case of nitrofurantoin, the interaction of the free electron with the nitrogens and protons gives a complex hyperfine pattern that has been analyzed and indicates the shift-base is intact (23, 24). In summary, the ESR spectrum shows that the free radical is simply nitrofurantoin plus an extra electron.

When we examined the effect of 100 μM nitrofurantoin on the NADPH-supported oxygen consumption by hepatic or pulmonary microsomes, we found that, indeed, the rate of oxygen uptake was increased sevenfold over the basal rate and that this stimulation was partially reversed by superoxide dismutase (Fig. 8). Again, the presence of superoxide anion radical strongly suggested that the nitrofurantoin anion free radical is formed by microsomal nitroreductase under aerobic conditions (25). As expected, the disproportionation of hydrogen peroxide by catalase also decreased the nitrofurantoin-stimulated oxygen uptake (Fig. 7). When both superoxide dismutase and catalase were added to the incubations, the nitrofurantoin-catalyzed oxygen consumption was decreased by over a third.

We have examined the paraquat-stimulated uptake of oxygen by microsomes in order to compare it with the nitrofurantoin-stimulated uptake (25). Paraquat stimulates the uptake of oxygen by microsomes less than an equal concentration of nitrofurantoin (Fig. 8). Otherwise, the effect of superoxide dismutase and/or catalase is similar to that observed with the nitrofurantoin-stimulated oxygen uptake (25).

| Table 1. The nitro compounds |
|-----------------------------|
| **Compound** | **Structure** |
| Nitrofurazone | ![Structure of Nitrofurazone](image-url) |
| Nitrofurantoin | ![Structure of Nitrofurantoin](image-url) |
| Benznidazole | ![Structure of Benznidazole](image-url) |
| p-Nitrobenzoate | ![Structure of p-Nitrobenzoate](image-url) |
| Metronidazole | ![Structure of Metronidazole](image-url) |

![Figure 8. The effect of superoxide dismutase and catalase on the stimulation of rat hepatic microsomal consumption of oxygen by nitrofurantoin and paraquat. Data from Mason and Holtzman (25).](image-url)
Our work on the effect of superoxide dismutase and catalase on the nitro compound-stimulated oxygen consumption by microsomes is consistent with the formation of nitroaromatic anion radicals under aerobic conditions, and the rapid air oxidation of these radical intermediates resulting in the catalytic generation of superoxide and the well-known oxygen inhibition of nitroreductases. We propose that the nitrofurantoin-catalyzed reduction of oxygen to superoxide and hydrogen peroxide may be responsible for some of the toxic manifestations that occur during nitrofurantoin therapy (25). For instance, we noted that the occasional cases of pulmonary edema and fibrosis caused by nitrofurantoin therapy are similar to the effects of paraquat poisoning. Subsequent work with animal models supported our proposal (26).

**Electron Transfer Theory**

These qualitative ideas of electron transfer can be expressed as a quantitative correlation. Wardman (27) has proposed that the reduction potential, $E_1^0$ (1-electron potential in water at pH 7), of nitro aromatic compounds is the most appropriate index of the redox properties of nitroaromatic compounds because it is the thermodynamic parameter that characterizes the relative ease of reduction of these compounds (27).

The thermodynamics of electron-transfer reactions involving free-radical intermediates are characterized by the difference in reduction potentials between the electron donor, the nitroreductase, and the acceptor, the nitro compound (28). However, only the equilibrium constant, $K_1$, can be calculated from a knowledge of the electrochemical potentials and, in general, the rate of approach to equilibrium can be negligibly slow even though the reaction is thermodynamically favorable. The quantum mechanical Marcus theory of electron-transfer reactions says that the rate constant $k_1$ can be related to the equilibrium constant $K_1$ (i.e., $\Delta E_1^0$) by the Marcus relationship for simple outer sphere electron transfer $k_1 = 4\pi \exp(-\Delta G^*/RT)$ where $A$ is a collision number and $\Delta G^*$ defined in its simplest form is related to the free energy $\Delta G$ and $\lambda$, which is a reorganization parameter for the water of solvation. Since $\Delta G^*$ is defined by $\Delta E_1^0$ for a one-electron transfer reaction, if the individual reduction potentials are known, then the rate constant $k_1$ as well as the equilibrium constant $K_1$ can be predicted. Over small ranges in $\Delta E_1^0$, log $k_1$ is proportional to $E_1^0$ (27).

To establish a correlation between the one-electron reduction potentials of nitro aromatic compounds with the kinetic parameters of a nitroreductase enzyme as measured by the rate of oxygen consumption, two nitroreductase enzymes were chosen for this study, ferredoxin:NADP+ oxidoreductase and NADPH-cytochrome P-450 reductase. The former was chosen because of its potent nitroreductase activity, and the latter was chosen because it is ubiquitous in mammalian cells (29). $K_m$ and $V_{max}$ for the ferredoxin:NADP+ oxidoreductase-nitroaromatic systems were determined from the rate of oxygen consumption, taken as the initial slope, using a calibrated Clark electrode (Table 2).

The enzyme kinetic parameters $V_{max}$ and $K_m$ were calculated utilizing the Lineweaver-Burk linearization (double-reciprocal plot) of the Michaelis-Menten equation. For nitrofurantoin there is a significant difference in the $V_{max}$ but no significant effect on the value of the $K_m$ when catalase and superoxide dismutase are present. Superoxide dismutase and catalase have little effect on the $V_{max}$ and $K_m$ values of the other nitro compounds (29). Neither $K_m$, log$K_m$, $V_{max}$, nor log$V_{max}$ correlated well with the reduction potentials (Table 2), although the trends are generally in the right direction with the notable exception of metronidazole; that is, as the compound gets harder to reduce, the $V_{max}$ gets smaller and the $K_m$ gets larger (Table 3). On the other hand, log$V_{max}/K_m$ consistently decreases as the reduction potential becomes more negative. Again, with NADPH-cytochrome P-450 reductase, no good correlation with $K_m$ or $V_{max}$ alone could be found, but log of $V_{max}/K_m$ consistently becomes smaller as the reduction potential becomes more negative (Table 3).

The plot of log($V_{max}/K_m$) versus the reduction potential in the ferredoxin reductase system gives a nearly

| Compound       | $V_{max}$, $\mu$mole O$_2$/mg/min | $K_m$, mM | log($V_{max}/K_m$) | $E_1^0$, V |
|----------------|----------------------------------|----------|-------------------|------------|
| Nitrofurazone  | 31.2 ± 3.7                       | 0.67 ± 0.09 | 1.67             | -0.257     |
| Nitrofurantoin | 49.0 ± 2.5                       | 1.3 ± 0.2  | 1.58              | -0.264     |
| Benznidazole   | 4.1 ± 1.0                        | 7.4 ± 2.2  | 0.25              | -0.380     |
| p-Nitrobenzene | 2.4 ± 0.2                        | 9.0 ± 0.1  | -0.57             | -0.415     |
| Metronidazole  | 3.9 ± 0.8                        | 139 ± 30   | -1.52             | -0.436     |

Table 3. Kinetic parameters for NADPH-cytochrome P-450 reductase with selected nitro aromatic compounds (29).

| Compound       | $V_{max}$, $\mu$mole/unit/min | $K_m$, mM | log($V_{max}/K_m$) | $E_1^0$, V |
|----------------|--------------------------------|----------|-------------------|------------|
| Nitrofurazone  | 55.4 ± 0.4                     | 0.10 ± 0.01 | -1.27            | -0.0327    |
| Nitrofurantoin | 25.3 ± 2.4                     | 0.24 ± 0.06 | -2.00            | -0.384     |
| Benznidazole   | 9.4 ± 1.9                      | 1.31 ± 0.25 | -3.14            | -0.380     |
| p-Nitrobenzene | 1.8 ± 0.6                      | 3.9 ± 1.9  | -4.33             | -0.415     |
| Metronidazole  | 9.6 ± 3.9                      | 78.5 ± 45.0 | -4.91            | -0.486     |

* Unit of cytochrome c assay $\times 10^4$.
perfect correlation (Fig. 9). The $V_{\text{max}}/K_m$ ratio is considered a measure of the enzyme-nitro substrate reactivity and of all consequent reactions that follow. In other words, the $V_{\text{max}}/K_m$ value is a measure of the enzyme's commitment to catalyze nitro reduction. Notice that a tenth of a volt change in reduction potential causes over an order of magnitude change in $V_{\text{max}}/K_m$.

NADPH-cytochrome P-450 reductase gives a similar correlation (Fig. 10). The slope of the line defined by the equation in Figure 10 is analogous to the redox dependence of a simple chemical rate constant and can be taken as a measure of the redox dependence correlating biological reductions with $E^\circ_1$. Wardman and Clark (30) have summarized some of the numerous redox correlations for nitro compounds (Tables 4 and 5). Considering the diversity of these studies, the close quantitative agreement with redox dependences around 10 V$^{-1}$ are striking. This coefficient defines an order of magnitude decrease in the concentration required to achieve a fixed response for an increase in $E^\circ_1$ of 0.1 V.

Note that cytotoxicity (Table 5) and the rate of reduction of nitro aromatic compounds (Table 4) have similar redox dependencies. With this in mind, it is not surprising that metronidazole, with the lowest reduction potential of any nitro drug, is also the safest nitro drug.

The traditional mechanism for cytotoxicity, mutagenicity, etc., of nitro compounds is that a reduction product such as the nitroso (the 2-electron reduction product) or hydroxylamine (the 4-electron reduction product) binds to DNA and leads ultimately to the biological response. It is quite reasonable that nitro anion formation is rate-limiting in the production of DNA damaging products, whatever they may be. This approach can be extended for quinone, bipiridylium, andazo compounds. Work is in progress in our laboratory on the $V_{\text{max}}$ and $K_m$ dependencies of bipiridylium and quinone compounds.

Table 4. Redox dependence of nitro aromatic compounds upon reduction by a variety of systems.

| Reduction by | Redox dependence, $V^{-1}$ |
|-------------|-----------------------------|
| Aerobic bacteria | 8.2$^a$ |
| Anaerobic mammalian cells | 10.7$^b$ |
| Anaerobic microsomes | 10.5$^c$ |
| Reduced flavin mononucleotide | 18.4$^d$ |
| Xanthine/xanthine oxidase | 13.8$^e$ |
| Ferredoxin:NADP$^+$ oxidoreductase | 13.5$^e$ 13.9$^e$ |
| NADPH-cytochrome P-450 reductase | 14.9$^e$ |

$^a$ d(log k)/d ($E^\circ$).
$^b$ Data taken from Wardman and Clark (30).
$^c$ Data taken from Orna and Mason (39).
$^d$ With catalase and superoxide dismutase.

Table 5. Cytotoxicity and mutagenicity redox dependence of nitroaromatic compounds.$^c$

| Toxicity | Redox dependence$^b$ |
|----------|-------------------|
| Cytotoxicity | |
| Bacteria | 11.5 |
| Anaerobic mammalian cells | 10.1 |
| Aerobic mammalian cells | 8.7 |
| Mutagenicity | |
| Aerobic bacteria | 11.2 |
| Aerobic mammalian cells | 7.4 |
| DNA synthesis | 12.5 |
| DNA strand breakage | 9.8 |
| DNA.$^a$ release of dT | 11 |

$^c$ Data taken from Wardman and Clarke (30).
$^b$ Mean of one to three studies.

REFERENCES

1. 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. Philpot, Eds.), Elsevier, New York, 1979, pp. 151–200.
2. Mason, R. P., and Chignell, C. F. Free radicals in pharmacology and toxicology-selected topics. Pharmacol. Rev. 33: 189–211 (1981).

3. 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.

4. Kappus, H., and Sies, H. Toxic drug effects associated with oxygen metabolism. Redox cycling and lipid peroxidation. Experientia 37: 1233–1241 (1981).

5. Michaelis, L., and Hill, E. S. Potentiometric studies on semiquinones. J. Am. Chem. Soc. 55: 1481–1484 (1933).

6. Homer, R. F., Mees, G. C., and Tomlinson, T. E. Mode of action of dipyridy l quarternary salts as herbicides. J. Sci. Food Agric. 11: 309–315 (1960).

7. Dodge, A. D. The mode of action of the bipyritydyl herbicides, parquat and diquat. Endeavour 30: 130–135 (1971).

8. Harbour, J. R., and Bolton, J. R. Superoxide formation in spinach chloroplasts: electron spin resonance detection by spin trapping. Biochem. Biophys. Res. Commun. 64: 830–837 (1977).

9. Gage, J. C. The action of parquat and diquat on the respiration of liver cell fractions. Biochem. J. 109: 757–761 (1968).

10. Rose, M. S., and Smith, L. L. Tissue uptake of parquat and diquat. Gen. Pharmacol. 8: 173–176 (1977).

11. Horton, J. K., Brigelius, R., Mason, R. P., and Bend, J. R. Paraoxidase uptake into freshly isolated rabbit lung epithelial cells and its reduction to the paraoxidase radical under anaerobic conditions. Mol. Pharm. 28: 484–488 (1986).

12. Rao, D. N. R., and Mason, R. P. One-electron reduction of paraquat and related bipyritydyl compounds by rat hepatocytes. Submitted.

13. Powis, G. Free radical formation by antitumor quinones. Free Rad. Biol. Med. 6: 63–101 (1989).

14. Gant, T. W., Rao, D. N. R., Mason, R. P., and Cohen, G. M. Redox cycling and sulphadiyl arylation; their relative importance in the mechanism of quinone cytotoxicity to isolated hepatocytes. Chem.-Biol. Interact. 65: 157–173 (1988).

15. Rao, D. N. R., Takahashi, N., and Mason, R. P. Characterization of a glutathione conjugate of the 1,4-benzosemiquinone-free radical formed in rat hepatocytes. J. Biol. Chem. 263: 17981–17986 (1988).

16. Handa, K., and Sato, S. Generation of free radicals of quinone group-containing antitumor chemicals in NADPH-microsome system as evidenced by initiation of sulfite oxidation. Gann 66: 43–47 (1975).

17. Handa, K., and Sato, S. Stimulation of microsomal NADPH oxidation by quinone-group-containing antitumor chemicals. Gann 67: 523–528 (1976).

18. Schreiber, J., Mottley, C., Sinha, B. K., Kalyanaraman, B., and Mason, R. P. One-electron reduction of daunomycin, daunomycinone, and 7-deoxydaunomycinone by the xanthine/xanthine oxidase system: detection of semiquinone free radicals by electron spin resonance. J. Amer. Chem. Soc. 109: 346–351 (1987).

19. Mason, R. P., Peterson, F. J., and Holtzman, J. L. The formation of an azo anion free radical metabolite during the microsomal azo reduction of sulfonazo III. Biochem. Biophys. Res. Commun. 75: 532–540 (1977).

20. Mason, R. P., Peterson, F. J., and Holtzman, J. L. Inhibition of azoreductase by oxygen. The role of the azo anion free radical metabolite in the reduction of oxygen to superoxide. Mol. Pharmacol. 14: 665–671 (1978).

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

22. Mason, R. P., and Holtzman, J. L. The mechanism of microsomal and mitochondri al nitroreductase. Electron spin resonance evidence for nitroaromatic free radical intermediates. Biochemistry 14: 1626–1632 (1975).

23. Rao, D. N. R., Harman, L., Motten, A., Schreiber, J., and Mason, R. P. Generation of radical anions of nitrofurantoin, misonidazole, and metronidazole by ascorbate. Arch. Biochem. Biophys. 255: 419–427 (1987).

24. Rao, D. N. R., Jordan, S., and Mason, R. P. Generation of nitro radical anions of some 5-nitrofurans, and 2- and 5-nitroimidazoles by rat hepatocytes. Biochem. Pharmacol. 37: 2907–2913 (1988).

25. 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).

26. Peterson, F. J., Combs, G. F., Jr., Holtzman, J. L., and Mason, R. P. Effect of selenium and vitamin E deficiency on nitrofurantoin toxicity in the chick. J. Nutr. 112: 1741–1746 (1982).

27. Wardman, P., and Wilson, I. Some reactions and properties of nitro radical-anions important in biology and medicine. Environ. Health Perspect. 64: 309–320 (1985).

28. Wardman, P. Control of the generation and reactions of free radicals in biological systems by kinetic and thermodynamic factors. Free Rad. Res. Commun. 2: 223–232 (1987).

29. Orna, M. V., and Mason, R. P. Correlation of kinetic parameters of nitroreductase enzymes with redox properties of nitroaromatic compounds. J. Biol. Chem., 264: 12379–12384 (1989).

30. Wardman, P., and Clarke, E. D. Electron transfer and radical-addition in the radiosensitization and chemotherapy of hypoxic cells. In: New Chemo and Radio sensitzing Drugs (A. Breccia and J. F. Fowler, Eds.), Lo Scarabeo, Bologna, 1985, pp. 21–98.