Volume 307, number 1, 108–112 FEBS 11207 July 1992

Minireview

Biologically relevant metal ion-dependent hydroxyl radical generation

An update

Barry Halliwell[a] and John M.C. Gutteridge[b]

[a]*Pulmonary-Critical Care Medicine, U.C. Davis Medical Center, 4301 X Street, Sacramento, CA 95817, USA* and [b]*Oxygen Chemistry Laboratory, Department of Anesthesia and Intensive Care, Royal Brompton Hospital, National Heart and Lung Institute, Sydney Street, London SW3 6LY, UK*

Received 11 May 1992

Transition metal ions, especially iron, appear to be important mediators of oxidative damage in vivo. Iron(II) reacts with H_2O_2 to give more-reactive radicals. On the basis of ESR spin-trapping data with DMPO, supported by aromatic hydroxylation studies and patterns of DNA base modification, it is concluded that hydroxyl radical ($OH^\bullet$) is likely to be the major damaging species formed in Fenton Systems under biologically-relevant conditions (which include iron concentrations no higher than the micromolar range). Although reactive oxo-iron species (such as ferryl and perferryl) may also be important, direct chemical evidence for their formation and identity in biologically relevant Fenton systems is currently lacking. Studies at alkaline pH values show that iron(IV) and iron(V) species are highly oxidizing under those reaction conditions, with a pattern of reactivity different from that of $OH^\bullet$.

Iron; Copper; Hydroxyl radical; Fenton reaction; DNA damage; Superoxide; Hydrogen peroxide

1. HYDROXYL RADICAL EXISTS

Hydroxyl radical[1], $OH^\bullet$, is produced when water is exposed to ionizing radiation and its properties have been well documented by radiation chemists (e.g. [1]). Hydroxyl radical reacts at, or close to, a diffusion-controlled rate with almost all biological molecules. Hence any $OH^\bullet$ formed in vivo will react with whatever is present at its site of formation, which makes it difficult to trap $OH^\bullet$ and demonstrate its formation directly in biological systems [2]. Attack of $OH^\bullet$ upon DNA produces a multiplicity of different products (1,3,4]; for example, all four purine and pyrimidine bases are modified [4,5]. By contrast, other oxygen-derived species either do not react with DNA at all (examples being superoxide radical, $O_2^{\bullet-}$, and hydrogen peroxide) or they preferentially or exclusively attack guanine (such as singlet O_2 and peroxyl radicals) [5]. The existence of repair systems which recognize many of these DNA-base lesions [6] and the excretion of a wide range of oxidatively-modified DNA bases in human urine [7,8] provide good evidence consistent with the view that $OH^\bullet$ is produced in vivo and attacks DNA (and presumably other molecules as well). Of course, such data do not explain how the $OH^\bullet$ arises: some may be generated by background radiation.

Abbreviations: DMPO, 5,5-dimethyl-1-pyrroline-*N*-oxide; DETAPAC, diethylenetriaminepentaacetic acid.

[1]Hydroxyl radical is often written $^\bullet OH$, to emphasize that the unpaired electron is located in the oxygen atom.

Correspondence address: B. Halliwell, Pulmonary-Critical Care Medicine, U.C. Davis Medical Center, 4301 X Street, Sacramento, CA 95817, USA. Fax: (1) (916) 734 7924.

2. SUPEROXIDE AND HYDROGEN PEROXIDE

As a result of the pioneering work of Chance et al. [9], Fridovich et al. [10] and others (reviewed in [2]), it has become well established that superoxide radical ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) are produced in vivo, although the exact amount of these species produced in mammals is still uncertain. Their generation can be accidental (e.g. production of $O_2^{\bullet-}$ by 'leakage' of electrons from electron transport chains [10]) or purposeful (e.g. $O_2^{\bullet-}$ production by activated phagocytic cells [11] and possibly by some other cell types, such as lymphocytes [12], endothelial cells [13] and fibroblasts [14]). Experimental manipulations of antioxidant defence enzymes have shown clearly that removal of most $O_2^{\bullet-}$ and H_2O_2 is essential in vivo [10,15]. These enzymes act as a coordinated system: hence, for example, too much superoxide dismutase can be deleterious [16]. Antioxidant defences seem to be approximately in balance with generation of oxygen-derived species in vivo. There seems to

Published by Elsevier Science Publishers B.V.

be no great reserve of antioxidant defence (although defences can often be induced in response to increased oxidative stress [10,17]), perhaps because some oxygen-derived species are useful [3,12–14,18].

Why is it biologically necessary to remove most $O_2^{\bullet-}$ and H_2O_2? Superoxide is a poorly reactive species [19]. A few metabolically-important enzymes can apparently be inactivated by $O_2^{\bullet-}$ in *E. coli* [20], but no important molecular targets of direct attack by $O_2^{\bullet-}$ have yet been identified in mammalian cells. The protonated form of $O_2^{\bullet-}$, perhydroxyl radical ($HO_2^{\bullet}$), is much more reactive than $O_2^{\bullet-}$ itself in vitro [19], but has not yet been shown to mediate any damaging effects in vivo. H_2O_2 at low (physiological) levels appears to be similarly unreactive. Indeed, its limited ability to oxidize critical -SH groups on proteins might even have some role in regulation of gene expression and metabolic activity [2,17,21].

Beauchamp and Fridovich [22] proposed that the toxicity of $O_2^{\bullet-}$ and H_2O_2 could involve their conversion into the much more reactive $OH^{\bullet}$. Two mechanisms have been proposed to explain this conversion. The more recent [23] is an interaction between $O_2^{\bullet-}$ and nitric oxide ($NO^{\bullet}$), a vasodilator radical produced by several cell types, including phagocytes and vascular endothelial cells.

$$NO^{\bullet} + O_2^{\bullet-} \rightarrow ONOO^- \text{ (peroxynitrite)} \quad (1)$$
$$ONOO^- + H^+ \rightarrow ONOOH \quad (2)$$
$$ONOOH \rightarrow OH^{\bullet} + NO_2^{\bullet} \quad (3)$$

Such a reaction has been demonstrated to occur in vitro [23,24] and its physiological significance is under intense investigation. For example, peroxynitrite might preferentially react with -SH groups or ascorbate instead of decomposing to give $OH^{\bullet}$, and $NO^{\bullet}$ has been suggested to have antioxidant effects in some systems [25,26], e.g. by removing $O_2^{\bullet-}$ [26,27].

An earlier mechanism proposed to explain $OH^{\bullet}$ formation was the metal ion-catalyzed Haber-Weiss reaction (reviewed in [28]).

$$O_2^{\bullet-} + H_2O_2 \xrightarrow{Fe/Cu} OH^{\bullet} + OH^- + O_2 \quad (4)$$

Most attention has been focused on iron as a catalyst, but interest in copper is increasing [28].

3. FENTON CHEMISTRY

In reaction 4, $OH^{\bullet}$ is usually suggested to be produced by reaction of reduced metal ions with H_2O_2, e.g. in the case of iron by the so-called Fenton reaction.

$$Fe^{2+} + H_2O_2 \rightarrow Fe\ (III) + OH^{\bullet} + OH^- \quad (5)$$

However, there has been repeated controversy as to whether $OH^{\bullet}$ is formed at all in Fenton reactions at physiological pH values. Challenges to the existence of $OH^{\bullet}$ seem to erupt every few years and then subside, leaving us none the wiser [28–36]. The authors believe that $OH^{\bullet}$ is formed in Fenton systems, but that other reactive species, presumably oxo-ion complexes, might be formed as well, although they remain to be characterized chemically [28,32]. Thus we view any oxo-iron species that may exist as additional to $OH^{\bullet}$, not alternatives to it [28].

Before discussing criteria for demonstrating the formation of $OH^{\bullet}$ in biologically relevant Fenton systems, let us review what is known about the chemistry of oxo-iron complexes that might be formed in simple Fenton systems. This will not take long: although there is a plethora of excellent literature identifying oxo-iron species at the active sites of haem-containing enzymes and proteins, almost nothing concrete is known about whether species such as ferryl (iron (IV)) and perferryl (iron (V)) can even exist in simple aqueous solutions of iron compounds at physiological pH. Even highly respected chemists have postulated the existence of reactive oxo-iron species as alternatives to $OH^{\bullet}$ in Fenton reactions on the mere basis of anomalies in kinetics or formation of 'unexpected' end products from substrates added to Fenton systems, without providing any direct (e.g. ESR, NMR, isolation) evidence for the existence of such species. Walling [32], Fitchett et al. [33], the authors [28], Yamazaki and Piette [34] and Croft et al. [35] have emphasized the dangers of misinterpretation in such approaches. Iron ions react not only with H_2O_2, but also with the initial products of attack of $OH^{\bullet}$ upon organic molecules. For example, Fe(III) can react with α-alcohol radicals formed by attack of $OH^{\bullet}$ upon alcohols. Claims by some authors that the 'oxidizing species' formed in Fenton systems reacts too slowly with *tert*-butanol (2-methylpropan-2-ol) to be $OH^{\bullet}$ might be accounted for by reduction of the $^{\bullet}CH_2C(CH_3)_2OH$ radical by Fe(II) [32,35].

Fortunately, Bielski et al. [36,37] are beginning to establish the precise chemistry of oxo-ion species. They start with a well-defined inorganic iron compound, potassium ferrate (K_2FeO_4), which contains Fe(VI). Solutions of this are stable for several hours at pH values above 8–9. One-electron reduction of ferrate(VI) yields an Fe(V) species, ferrate(V). Fe (IV) can be generated by oxidation of Fe(III) or, perhaps, by one-electron reduction of Fe(V). Fe(IV), and especially Fe(V), were found to be much more reactive in aqueous solution than is Fe(VI). For example, Fe(V) reacts with formate 10^5 times faster than Fe(VI), 10–100 times faster with ascorbate [36] and 10^3–10^5 times faster with amino acids [37]. These hypervalent iron species apparently prefer to react with most amino acids at either the α-carbon or the α-nitrogen, whereas $OH^{\bullet}$ tends to react with all sites indiscriminately. Indeed, in the case of aromatic amino acids, reaction of $OH^{\bullet}$ with the benzene ring is often the preferred reaction pathway [32,38] whereas Fe(V) preferentially attacks the side chain. Of course, one must be

wary of comparing the reactivity of oxo-iron species at highly alkaline pH values with those of $OH^{\bullet}$ at pH 7.4.

Bearing in mind that they are conducted at highly alkaline pH values, the studies of Bielski et al. [36,37] are important because they provide guidelines as to the possible reactivity of any oxo-iron complexes that are generated in Fenton systems at pH 7.4. They also suggest methods by which Fe(V) and Fe(IV) might be distinguished from $OH^{\bullet}$ (e.g. by examining the pattern of products formed from aromatic ring structures). For example, if the reactivity of ferrate(V) at alkaline pH were representative of the proposed perferryl species at physiological pH, then assumptions [28] that perferryl is a fairly unreactive agent (based on the low reactivity of the perferryl forms of enzymes such as horseradish peroxidase) may have been in error. Of course, comparison of these ferrate(V) and ferrate(IV) species with ferryl/perferryl in enzymes must consider not only the different pH values employed but also the great chemical differences between a metalloprotein with nitrogenous and other ligands to the metal and a simple inorganic oxo-iron compound.

4. APPROACHES AND ASSUMPTIONS IN DEMONSTRATING HYDROXYL RADICAL IN FENTON SYSTEMS

In order to demonstrate unequivocally that $OH^{\bullet}$ is produced in Fenton reactions, what is needed is a method that detects $OH^{\bullet}$ and nothing else. Many authors believe that spin trapping is such a definitive method [33,35,39–41], especially when DMPO is used as a trap, with appropriate controls to rule out artefacts due to the reaction of DMPO with $O_2^{\bullet-}$. Thus, Yamazaki and Piette [39] state that 'there is no doubt that the ESR spin-trapping technique is the most direct method to measure $OH^{\bullet}$'. Determination of the pattern of end products of attack of reactive species upon aromatic compounds may also be useful for detecting $OH^{\bullet}$ [38]. However, the isomeric distribution of end products is markedly affected by the composition of the reaction mixture [32,38] and singlet oxygen can hydroxylate aromatic rings, although it appears to give a more restricted range of products than does $OH^{\bullet}$ [42]. The pattern of chemical modification of purine and pyrimidine bases when DNA is exposed to Fenton systems has also been proposed to be diagnostic for formation of $OH^{\bullet}$ [5], although at least one of these products, 8-hydroxyguanine, can be generated by attack of other species upon guanine (reviewed in [5]).

Yamazaki and Piette [39] used spin trapping with DMPO to study the reactions of Fe^{2+}–DETAPAC, Fe^{2+}– EDTA and Fe^{2+}–ADP with H_2O_2 at pH 7.4. They concluded that, at low (<1 μM) Fe^{2+} concentrations, $OH^{\bullet}$ was formed almost quantatively according to equation 5. At higher Fe^{2+}–EDTA or Fe^{2+}–ADP concentrations, the stoichiometry changed and the results were explained on the basis of formation of both $OH^{\bullet}$ and of additional oxidizing species (although, again, no direct evidence for existence of these species was presented).

If we accept the spin-trapping data [33–35, 39–41], it is clear that $OH^{\bullet}$ is formed in Fenton reactions at pH 7.4, a result supported by aromatic hydroxylation studies [38] and patterns of DNA base modification [5]. However, it must be noted that there is no direct proof that DMPO reacts only with $OH^{\bullet}$. For example, it might react with ferryl,

$$Fe(IV)=O+DMPO+H^{+}\rightarrow Fe(III)+DMPO\text{–}OH \quad (6)$$

and/or with other oxo-iron species. Indeed, the DMPO–OH adduct may be produced by reaction of the spin trap with hypochlorous acid or singlet O_2 [66,67], so it is clearly not specific for $OH^{\bullet}$. Similarly, ferryl and/or perferryl species might be able to hydroxylate aromatic compounds and oxidize DNA bases, although the DNA-cleaving bleomycin-oxo-iron species (thought to be a perferryl or ferryl bleomycin [43]) does not form DNA base oxidation products of the type that $OH^{\bullet}$ can generate [44]. Oxo-iron species might also be able to react with some or all of the commonly used $OH^{\bullet}$ scavengers, but there is considerable disagreement on this point between the papers postulating such species. For example, some studies claim that 'non-$OH^{\bullet}$ species' generated in Fenton reactions do not react with *tert*-butanol [29,45] whereas others claim that they do [34].

Apart from this fundamental question as to what is measured, there are several other concerns in applying spin-trapping techniques to biological Fenton systems [40]. Many of the reducing agents that recycle Fe(II) interfere with the spin-radical adduct. Thus, ascorbate can reduce it to an ESR-silent species and so, ascorbate may appear to be an antioxidant when it is actually accelerating $OH^{\bullet}$ formation (Gutteridge and McCay, unpublished data). Another consideration is that spin traps may have to compete with biological molecules for binding the iron needed to drive site-specific biological Fenton chemistry. Indeed, the design of spin traps with metal-binding ability is an interesting approach for the future.

In the view of the authors, progress will only be made when the oxo-iron species are characterized chemically and their reactions with DMPO, aromatic compounds and DNA are studied directly, and compared with those of $OH^{\bullet}$. The existence of oxo-iron species cannot be established on the basis of anomalies in reaction kinetics or in formation of end products. The pioneering studies of Bielski et al. [36,37] need to be extended to biologically relevant conditions.

5. HOW MUCH FENTON-REACTIVE IRON EXISTS IN VIVO?

If we accept the spin-trapping data at face value, it

seems that $OH^{\bullet}$ is the major, if not the only, reactive species formed in Fenton chemistry at low Fe concentrations [33–35] although the ligand to the iron and the H_2O_2 concentration are also important variables [28,29,31,33,35,46]. How much 'catalytic' iron actually exists in vivo?

Organisms take great care in the handling of iron, using transport proteins such as transferrin and storage proteins such as ferritin and haemosiderin to minimize iron reactivity and availability (reviewed in [18,47]). Thus, for example, plasma from healthy human adults contains no 'free' iron capable of promoting Fenton chemistry (reviewed in [48]). The effectiveness of this iron ion sequestration may be illustrated not only by biochemical analysis [18,47,48], but also by a simple observation. Humans appear to be able to consume large quantities of ascorbic acid for years without ill effects. Indeed, ascorbate may have important antioxidant properties in vivo in healthy subjects [49]. Yet, in the presence of iron and copper ions, ascorbic acid accelerates oxidative damage towards DNA, lipids and proteins [2,5,18,28,50]. It follows that catalytic metal ions are available only to a very limited extent in vivo: indeed, safe 'sequestration' of transition metal ions is probably an important antioxidant defence in its own right [2,18,28,47], as well as allowing ascorbate to exert its antioxidant effects. In agreement with this conclusion, giving ascorbate to iron-overloaded patients who have non-transferrin-bound iron in their plasma can be deleterious [51].

This limited availability of transition metal catalysts of Fenton chemistry in vivo is supported by results obtained using the bleomycin assay, developed by Gutteridge et al. as a first attempt to measure the availability of iron complexes catalytic for free radical reactions in human body fluids (reviewed in [48]). Except in the special case of iron overload, iron concentrations measured in body fluids by the bleomycin assay are rarely greater than 1–2 μM, often less, and are zero in plasma from healthy humans [48]. Intracellular iron levels are uncertain, but may be in the same low range.

Thus, if 'catalytic' iron levels are very low in vivo, then any biologically relevant Fenton chemistry will operate under conditions that are reported to favour the formation of $OH^{\bullet}$ [34,39]. Levels of 'catalytic' copper are not well established, but the limited data available suggest that they are even lower than those of iron in humans [52,53]. Thus, as has been emphasized previously [2,18], experiments in vitro in which 50–200 μM concentrations of iron complexes are used to stimulate lipid peroxidation or $OH^{\bullet}$ generation are unrepresentative of the situation in vivo. That iron-dependent free radical reactions do nevertheless, occur in vivo and are important mediators of oxidative damage is supported by a wealth of evidence, as has been discussed by different scientists many times in the past decade (e.g. see [2,5,18,27,28,41,43,47,54–65]).

Acknowledgements: The authors are very grateful to Dr. Ben Bielski for his helpful comments and criticisms. Barry Halliwell thanks the Arthritis and Rheumatism Council, Cancer Research Campaign and Medical Research Council for support. J.M.C.G. thanks the British Lung Foundation for support.

REFERENCES

[1] Von Sonntag, C. (1987) The Chemical Basis of Radiation Biology, Taylor and Francis, London.
[2] Halliwell, B. and Gutteridge, J.M.C. (1989) Free Radicals in Biology and Medicine, Second Edition, Clarendon Press, Oxford.
[3] Steenken, S. (1989) Chem. Rev. 89, 503–520.
[4] Dizdaroglu, M. (1990) Methods Enzymol. 193, 842–857.
[5] Halliwell, B. and Aruoma, O.I. (1991) FEBS Lett. 281, 9–19.
[6] Breimer, L.H. (1991) Free Radical Res. Commun. 14, 159–171.
[7] Wagner, J.R., Hu, C.C. and Ames, B.N. (1992) Proc. Natl. Acad. Sci. USA 89, 3380–3384.
[8] Stillwell, W.G., Xu, H.X., Adkins, J.A., Wishnock, J.S. and Tannenbaum, S.R. (1989) Chem. Res. Tox. 2, 94–99.
[9] Chance, B., Sies, H. and Boveris, A. (1979) Physiol. Rev. 59, 527–605.
[10] Fridovich, I. (1989) J. Biol. Chem. 264, 7761–7764.
[11] Babior, B.M. and Woodman, R.C. (1990) Semin. Hematol. 27, 247–259.
[12] Maly, F.E. (1990) Free Radical Res. Commun. 8, 143–148.
[13] Babbs, C.F., Cregor, M.D., Turek, J.J. and Badylak, S.F. (1991) Lab. Invest. 65, 484–496.
[14] Murrell, G.A.C., Francis, M.J.O. and Bromley, L. (1990) Biochem. J. 265, 659–665.
[15] Touati, D. (1989) Free Radical Res. Commun. 8, 1–8.
[16] Amstad, P., Peskin, A., Shah, G., Mirault, M.E., Moret, R., Zbinden, I. and Cerutti, P. (1991) Biochemistry 30, 9305–9313.
[17] Tartaglia, L.A., Gimenos, C.J., Storz, G. and Ames, B.N. (1992) J. Biol. Chem. 267, 2038–2045.
[18] Halliwell, B. and Gutteridge, J.M.C. (1986) Arch. Biochem. Biophys. 246, 501–514.
[19] Bielski, B.H.J. and Cabelli, D.E. (1991) Int. J. Radiat. Biol. 59, 291–319.
[20] Gardner, P.R. and Fridovich, I. (1991) J. Biol. Chem. 266, 19328–19333.
[21] Fanburg, B.L., Massaro, D.J., Certutti, P.A., Gail, D.B. and Berberich, M.A. (1992) Am. J. Physiol. 262, L235–L241.
[22] Beauchamp, C. and Fridovich, I. (1970) J. Biol. Chem. 245, 4641–4646.
[23] Beckman, J.S., Beckman, T.W., Chen, J., Marshall, P.A. and Freeman, B.A. (1990) Proc. Natl. Acad. Sci. USA 87, 1620–1624.
[24] Hogg, H., Darley-Usmar, V.M., Wilson, M.T. and Moncada, S. (1992) Biochem. J. 281, 419–424.
[25] Kanner, J., Harel, S. and Granit, R. (1991) Arch. Biochem. Biophys. 289, 130–136.
[26] Rubanyi, G.M., Ho, E.H., Cantor, E.H., Lumma, W.C. and Botelho, L.H.P. (1991) Biochem. Biophys. Res. Commun. 181, 1392–1397.
[27] Halliwell, B. (1989) Free Radical Res. Commun. 5, 315–318.
[28] Halliwell, B. and Gutteridge, J.M.C. (1990) Methods Enzymol. 186, 1–85.
[29] Rush, J.D. and Koppenol, W.H. (1986) J. Biol. Chem. 261, 6730–6733.
[30] Maskos, Z. and Koppenol, W.H. (1991) Free Rad. Biol. Med. 11, 609–610.
[31] Gutteridge, J.M.C., Maidt, L. and Poyer, L. (1990) Biochem. J. 269, 169–174.
[32] Walling, C. (1975). Acc. Chem. Res. 8, 125–132.
[33] Fitchett, M., Gilbert, B.C. and Jeff, M. (1985) Phil. Trans R. Soc. Lond. B311, 517–529.
[34] Yamazaki, I. and Piette, L.H. (1991) J. Am. Chem. Soc. 113, 7588–759.

[35] Croft, S., Gilbert, B.C., Lindsay Smith, J.R. and Whitwood, A.C. (1992) Free Radical Res. Commun. in the press.
[36] Bielski, B.H.J. (1991) Free Radical Res. Commun. 12, 469–477.
[37] Sharma, V.K. and Bielski, B.H.J. (1991) Inorg. Chem. 30, 4306–4310.
[38] Halliwell, B., Gutteridge, J.M.C. and Grootveld, M. (1988) Methods Biochem. Anal. 33, 59–90.
[39] Yamazaki, I. and Piette, L.H. (1990) J. Biol. Chem. 265, 13589–13594.
[40] Finkelstein, E., Rosen, G.M. and Rauckman, E.J. (1980) Arch. Biochem. Biophys. 200, 1–16.
[41] Burkitt, M.J. and Mason, R.P. (1991) Proc. Natl. Acad. Sci. USA 88, 8440–8444.
[42] Feix, J.D. and Kalyanaraman, B. (1991) Arch. Biochem. Biophys. 291, 43–51.
[43] Petering, D.H., Byrnes, R.W. and Antholine, W.E. (1990) Chem.-Biol. Interac. 73, 133–182.
[44] Gajewski, E., Aruoma, O.I., Dizdaroglu, M. and Halliwell, B. (1991) Biochemistry 30, 2444–2448.
[45] Rahhal, S. and Richter, H.W. (1988) J. Am. Chem. Soc. 110, 3126–3133.
[46] Gutteridge, J.M.C., Zs-Nagy, I., Maidt, L. and Floyd, R.A. (1990) Arch. Biochem. Biophys. 277, 422–428.
[47] Halliwell, B. and Gutteridge, J.M.C. (1990) Arch. Biochem. Biophys. 280, 1–8.
[48] Gutteridge, J.M.C. and Halliwell, B. (1987) Life Chem. Rep. 4, 113–142.
[49] Stocker, R. and Frei, B. (1991) In Oxidative Stress, Oxidants and Antioxidants (H. Sies, Ed.), Academic Press, New York.
[50] Stadtman, E.R. and Oliver, C.N. (1991) J. Biol. Chem. 266, 2005–2008.
[51] Nienhuis, A.W. (1981) New Engl. J. Med. 304, 170–171.
[52] Gutteridge, J.M.C. (1984) Biochem. J. 218, 983–985.
[53] Evans, P.J., Bomford, A. and Halliwell, B. (1989) Free Radical Res. Commun. 7, 55–62.
[54] Aust, S.D., Morehouse, L.A. and Thomas, C.E. (1985) Adv. Free Rad. Biol. Med. 1, 3–25.
[55] Cochrane, C.G. (1991) Mol. Aspects Med. 12, 137–147.
[56] Imlay, J.A. and Linn, S. (1988) Science 240, 1302–1309.
[57] Wolff, S.P. (1987) in Diabetic Complications (M.J.C. Crabbe, Ed.), Churchill Livingstone, Edinburgh.
[58] Farber, J.L. (1990) Chem. Res. Tox. 3, 503–508.
[59] Ferrali, M., Ciccoli, L., Signorini, C. and Comporti, M. (1990) Biochem. Pharmacol. 40, 1485–1490.
[60] Britigan, B.E. and Edeker, B.L. (1991) J. Clin. Invest. 88, 1092–1102.
[61] Walker, P.D. and Shah, S.V. (1991) Kidney Int. 40, 891–898.
[62] Frank, L. (1991) Free Rad. Biol. Med. 11, 341–348.
[63] Mello-Filho, A.C. and Meneghini, R. (1991) Mut. Res. 251, 109–113.
[64] Bissett, D.L., Chatterjee, R. and Hannon, D.P. (1991) Photochem. Photobiol. 54, 215–223.
[65] Howard, R.L., Buddington, B. and Allfrey, A.C. (1991) Kidney Int. 40, 923–926.
[66] Sai, K., Uchiyama, S., Ohno, Y., Hasegawa, R. and Kurokawa, Y. (1992) Carcinogenesis 13, 333–339.
[67] Bernofsky, C., Bandara, B.M.R. and Hingosa, O. (1990) Free Rad. Biol. Med. 8, 231–239.