The use of electron-spin-resonance techniques to detect free-radical formation and tissue damage

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Free radicals are commonly defined as molecules or molecular fragments containing a single unpaired electron; the presence of the unpaired electron usually confers a considerable degree of chemical reactivity on such species, though this may vary dramatically with the radical under consideration. Although a number of stable free radicals are known (for example, diphenylpicrylhydrazyl and Fremy's salt (potassium nitrosodisulphonate)), most of the radicals of biological interest have considerable chemical reactivity and corresponding short half-lives; for example, the calculated half-lives of the hydroxyl radical ($HO^{\cdot}$) or trichloromethylperoxyl radical ($CCl_3O_2^{\cdot}$) in the liquid phase in a biological milieu are of the order of 1 ns and 1 μs respectively (Slater, 1984).

Such radicals can be produced in tissues by a wide variety of methods (see Table 1; Pryor, 1966; Slater, 1972) and it is this wide range of possible generating systems which underlies the considerable and widespread current interest in free-radical-mediated tissue injury (for reviews, see Slater, 1972, 1978, 1984; Pryor, 1976–86; Mason, 1982; Halliwell & Gutteridge, 1984, 1985). An abbreviated list of cellular injuries and diseases which have been shown to involve free-radical production as either a primary or secondary feature is given in Table 2. Nutritional factors can play an important part in the aetiology of many free-radical disturbances in two ways: (1) by altering the levels of naturally occurring protective systems (see Table 3) or (2) by increasing tissue levels of substances (such as some transition metal ions) which can stimulate radical production in some way (Slater *et al.* 1987).

Direct proof of radical production in a particular system is, however, very difficult to achieve due to the high reactivity and short half-lives of many of the species involved. In many cases the only evidence for the involvement of radicals arises from studies on the relatively long-term effects of such species, i.e. the detection of products which arise from radical reactions (malondialdehyde, conjugated dienes, lipid hydroperoxides etc., for methodology, see Packer, 1984). This is often unsatisfactory due to the possibilities of misinterpretation and the lack of information as to the time-course of events; radical

Table 1. *Mechanisms resulting in the formation of reactive free radicals*

(1) By the absorption of radiation:
  (a) γ-, X-ray or neutron radiation
  (b) ultraviolet radiation
  (c) visible light in the presence of photosensitizers
  (d) thermal degradation of organic compounds
(2) By electron transfer (redox) reactions:
  (a) catalysed by transition metal ions
  (b) catalysed by enzymes
  (c) involving strong oxidizing or reducing agents

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Table 2. *Diseases and toxic cell injuries that are associated with free-radical disturbances*

Nutritional disorders:	deficiences of antioxidants, deficiences of trace metals for protective enzymes, or excesses of radical-initiating agents
Inflammation	
Rheumatoid arthritis	
Atherosclerosis	
Some parasitic infections:	malaria
Some lung disorders:	paraquat poisoning, 3-methylindole toxicity, asbestos
Alcoholism	
Iron overload:	haemochromatosis, kwashiorkor
Toxic liver injury:	carbon tetrachloride and related halogeno-alkanes, bromobenzene, paracetamol, hydrazines
Some tumour promoters	
Some examples of chemical carcinogenesis	
Reperfusion injury:	heart, brain, liver, gut, lung, kidney, pancreas, skin

Table 3. *Protective agents and antioxidants*

Protective enzymes:	superoxide dismutases (*EC* 1.15.1.1)
	catalase (*EC* 1.11.1.6)
	glutathione peroxidase (*EC* 1.11.1.9)
	glutathione transferases (*EC* 2.5.1.18)
Lipid-soluble antioxidants:	α-tocopherol
	β-carotene
	retinoic acid
	ubiquinone
	bilirubin, biliverdin
Water-soluble antioxidants:	urate
	ascorbate
	glutathione
	protein thiols

generation may be the cause of a particular lesion, though it may also arise from the degradation of previously damaged cellular components, i.e. as a consequence of another injurious process.

Electron-spin-resonance (esr) spectroscopy is one of the few methods of directly detecting free-radical species in biological systems (Swartz *et al.* 1972). This technique relies on the absorbance of microwave radiation, which arises from the promotion of the unpaired electron to a higher energy state, when the sample is placed in a variable magnetic field. The values of the microwave frequency and magnetic field where this absorption occurs (*g* factor) are characteristic of the type of radical present in the system. This absorption band, which is usually recorded as its first derivative signal to improve spectral resolution, can be split into several components by the presence of other magnetic nuclei (i.e. those with nuclear spin (I) > 0) in the vicinity of the singly occupied orbital. The number and size of the splittings (hyperfine coupling constants) between these absorption bands can give very valuable information as to the identity and structure of the radical(s) present. As this technique only gives signals with species with unpaired electrons (and not even all of these, see p. 399) it is very specific and can even be used in situations where there are thousands of other (non-radical) compounds present.

Unfortunately from a biological viewpoint the lower limit for the detection of radicals is of the order of 10^{-8} M using existing instrumentation, though adequately to resolve

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spectral lines in aqueous biological samples (and thus obtain information about the identity of the species) the limit is significantly higher than this (10^{-6}–10^{-7} M). In addition to this constraint certain types of radical cannot be detected due to the broadness of their spectral lines; some biologically interesting radicals such as $HO^{\cdot}$, $O_2^{\cdot -}$, $RO^{\cdot}$ and $RS^{\cdot}$ are included in this category.

Direct detection of radicals by esr can therefore only be carried out when there is a high steady-state concentration of radicals, i.e. in systems with a rapid rate of radical generation or where the radicals are long lived, or both. Despite these constraints some very valuable information has been obtained about biologically important radicals such as flavins (Beinert, 1972), semiquinones (produced by one-electron reduction of quinones such as adriamycin and menadione; Yamazaki, 1977), phenothiazine radical-cations (produced by metabolism of drugs such as chlorpromazine; Borg, 1972), aryloxy radicals (Yamazaki, 1977), nitroxides (for example, those produced by oxidation of amines such as β-naphthylamine; Floyd, 1980), amino radicals (Mason, 1982), nitroaromatic radical-anions (formed by one-electron reduction of nitro compounds such as misonidazole and metronidazole which are used as radiosensitizers and antimicrobial agents; Mason, 1982) and peroxyl radicals (Benedetto *et al.* 1981; Kalyanaraman, 1982). This area of study has been well reviewed (Mason, 1982; Mason & Chignell, 1982).

If the radicals are undetectable due to effects such as line broadening, high reactivity, short half-lives or slow rates of radical production, certain special techniques such as spin-trapping, rapid freezing, lyophilization, and rapid-flow systems can be used. Only the first of these techniques will be discussed further here; general comments and reviews on the latter methods can be found in Swartz *et al.* (1972) and Davies (1987).

The spin-trapping method uses an exogenously added compound to trap the highly reactive radical and convert it into a more stable, detectable, radical adduct (reaction 1). This allows the progressive accumulation of the relatively stable radical adduct with time, allowing radicals produced at a slow rate to be detected. Most of the commonly used spin-traps (usually nitroxide or nitrone compounds; see Fig. 1) give nitroxyl-type adducts that have characteristic esr behaviour allowing at least some information to be obtained about the trapped species. These spin-traps, which can be used both in vitro and in vivo, have very different specificities in the radicals with which they give stable adducts; care must therefore be taken in choosing which spin-trap to use in a particular system. For example, to trap $HO^{\cdot}$ and $O_2^{\cdot -}$, the cyclic spin-traps 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) and 3,3,5,5-tetramethyl-1-pyrroline-N-oxide (TMPO) are the spin-traps of choice (Rosen & Finkelstein, 1985), as the other spin-traps illustrated in Fig. 1 do not give readily detectable spin adducts with these species. Several excellent reviews on this subject have appeared (see Finkelstein *et al.*1980; Janzen, 1980; Perkins, 1980; Kalyanaraman, 1982; Mason, 1984; Rosen & Finkelstein, 1985). The subsequent part of the present article will concentrate on the applications of esr to an example of radical-induced cellular damage.

$$X^{\cdot} + \text{spin-trap} \longrightarrow \text{spin adduct radical} . \qquad (1)$$

Lipid peroxidation induced by the metabolism of halocarbons

Carbon tetrachloride and related halogenated methanes and ethanes have long been known to be severe hepatotoxins in a wide variety of species, and the study of the mechanism(s) of tissue injury induced by this family of compounds has provided much valuable information as to the biological consequences of radical production (for reviews, see Slater, 1982; Cheeseman *et al.* 1985). In 1966 two papers appeared presenting hypotheses for the mechanism of hepatotoxicity of CCl_4 (Recknagel &

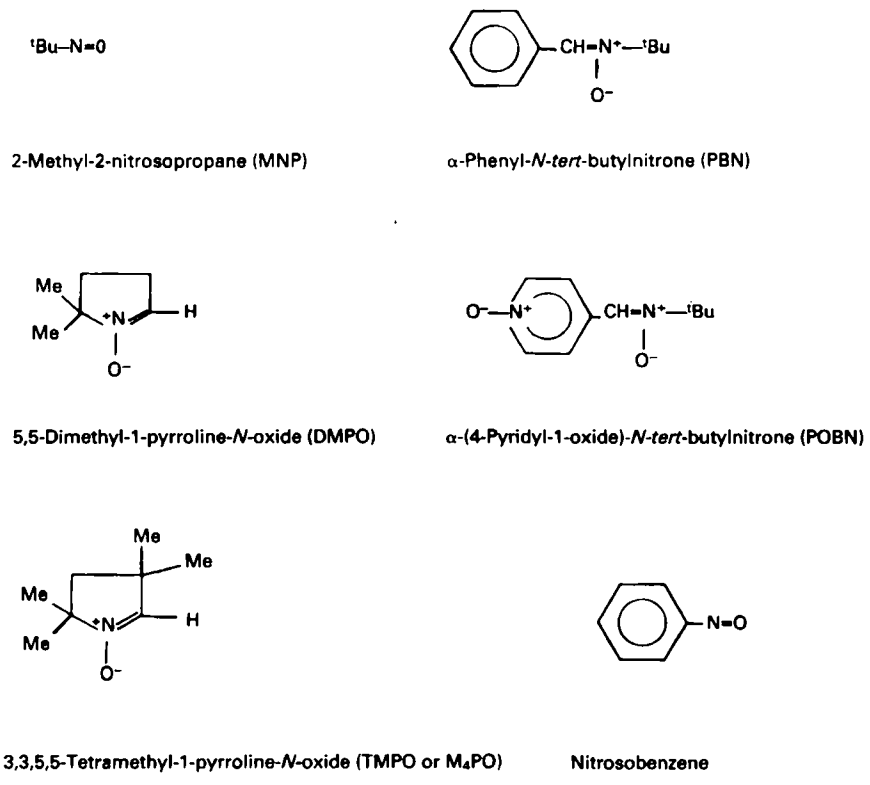

Fig. 1. Structural formulas of some commonly used spin-traps.

Ghoshal, 1966; Slater, 1966); each proposed the activation of CCl_4 to a free radical capable of initiating lipid peroxidation. Indirect studies by a number of groups (for review, see Cheeseman *et al.* 1985) confirmed this postulated mechanism, showing that the locus of activity appeared to be a particular isozyme of the cytochrome P_{450} system in the liver endoplasmic reticulum with the complete electron transport chain (i.e. NADPH, NADPH-cytochrome P_{450} reductase (*EC* 1.6.2.4) and the cytochrome P_{450} itself) required for full activity.

Direct proof of the involvement of $^{\cdot}CCl_3$ radicals produced by the postulated one-electron reduction–fragmentation process (reaction 2) was obtained in 1980 using esr spin-trapping and the spin-trap α-phenyl-*N*-*tert*-butylnitrone (PBN) (Poyer *et al.* 1980; Tomasi *et al.* 1980); previous studies (Ingall *et al.* 1978) using the spin-trap 2-methyl-2-nitrosopropane (MNP) had detected a radical adduct in a system undergoing CCl_4-induced lipid peroxidation, though the observed adduct was not directly derived from $^{\cdot}CCl_3$, and was later shown to be a lipid-derived species.

$$CCl_4 + e^- \longrightarrow CCl_4^{-\cdot} \rightarrow {}^{\cdot}CCl_3 + Cl^- \qquad (2)$$

Incubation of rat-liver microsomes with CCl_4 and an NADPH-generating system in the presence of PBN gave an esr spectrum which was dependent on the presence of all the components of this system (Fig. 2) The behaviour of this adduct is what would be

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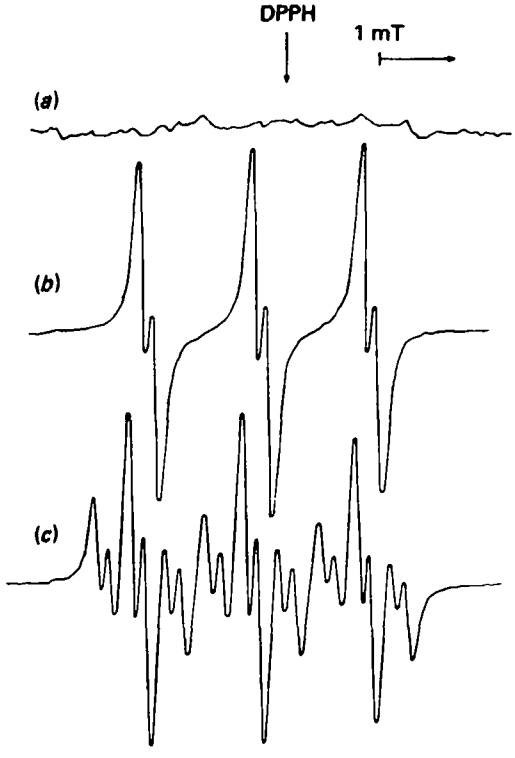

Fig. 2. Electron-spin-resonance spectra of the α-phenyl-*N-tert*-butylnitrone (PBN)-$CCl_3^{\cdot}$ spin adduct obtained from incubation (10 min, 37°) of isolated liver cells (10^7 cells/ml) with the spin-trap PBN (25mM) either alone (*a*), or with 10 μl carbon tetrachloride in the central well of the incubation flasks (*b*). Spectrum (*c*) was obtained in the same way as (*b*) except that the CCl_4 used was enriched (45%) with $^{13}CCl_4$. Identical spectra were obtained from (1) incubation of rat-liver microsomes with an NADPH-generating system in the presence of PBN and CCl_4, or (2) from lipid extracts of livers obtained from male rats injected intraperitoneally 15 min previously with 1 ml of a solution containing 17 mg PBN and 0·1 ml CCl_4 in maize oil (Albano *et al.* 1982). DPPH, diphenylpicrylhydrazyl radical.

expected for the PBN-CCl_3 adduct and is identical to that obtained for this radical adduct in model systems. Confirmation of the identity of this adduct was obtained (Poyer *et al.* 1980; Tomasi *et al.* 1980) by use of $^{13}CCl_4$, which gave a supplementary splitting in the observed spectrum due to the presence of the magnetic ^{13}C nucleus ($I = 1/2$). Similar signals were obtained not only in isolated liver cells exposed to CCl_4, but also in the intact livers of rats dosed with CCl_4 and the spin-trap (Albano *et al.* 1982).

Table 4. *Effects of lipid peroxidation on membrane structure and function*

(1) Decrease in relative content of polyunsaturated fatty acids (PUFA), especially $C_{20:4}$ and $C_{22:6}$
(2) Formation of lipid hydroperoxides that can stimulate or inhibit specific enzymes
(3) Oxidation of protein thiol groups which may affect enzyme activities and protein conformation
(4) Alterations in bilayer fluidity
(5) Liberation of breakdown products from the site of peroxidation to produce damaging effects elsewhere: malondialdehyde, saturated and unsaturated aldehydes, alkanes, epoxy- and hydroxy-fatty acids
(6) Changes in membrane-receptor structure and function.

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Kinetic information obtained from pulse radiolysis experiments have subsequently shown that the rates of reaction of the $^{\cdot}CCl_3$ radical with biological molecules are very slow, and it has been shown that the initially produced $^{\cdot}CCl_3$ species react rapidly with oxygen to give the corresponding peroxyl radical $CCl_3O_2^{\cdot}$ (Packer *et al.* 1978). This radical has been shown to react rapidly with a large number of biologically relevant molecules such as amino acids and unsaturated fatty acids, and may well be the ultimately damaging species at least with respect to the initiation of lipid peroxidation (Forni *et al.* 1983; Cheeseman *et al.* 1985). Despite numerous studies using a large number of spin-traps, this peroxyl radical has yet to be observed directly in a biological system (Cheeseman *et al.* 1985); this is due not only to its high reactivity with other molecules, but also to the instability of its adducts with spin-traps which decay rapidly.

Biological membranes (such as plasma membrane, endoplasmic reticulum, mitochondrial membrane, etc.) contain substantial quantities of polyunsaturated fatty acids (PUFA) which are highly susceptible to radical-induced peroxidative breakdown. The initial step in this process is hydrogen-atom abstraction (by, for example, $CCl_3O_2^{\cdot}$. or other xenobiotic-derived radicals) from a bis-methylene group of a PUFA molecule (reaction 3). This lipid radical then rapidly reacts with O_2 (at approximately diffusion-controlled rate) to give a lipid peroxyl radical (PUFA-OO$^{\cdot}$; reaction 4), which then reacts either with another lipid molecule to initiate a chain process (reaction 5) or with another component of the system to give a very wide variety of products.

This process has been the subject of numerous investigations (for example, see Dianzani & Ugazio, 1978; Dormandy, 1978; Bus & Gibson, 1979) and the biological consequences of these reactions elucidated (Table 4). Esr spin-trapping studies, using a wide variety of spin-traps, have yielded some valuable information about the mechanisms of these processes, and a wide variety of lipid-derived radicals have been detected; for a recent review of this area, see Davies (1987).

$$X^{\cdot} + PUFA \longrightarrow XH + PUFA^{\cdot}, \quad (3)$$

$$PUFA^{\cdot} + O_2 \longrightarrow PUFA\text{-}OO^{\cdot}, \quad (4)$$

$$PUFA\text{-}OO^{\cdot} + PUFA \longrightarrow PUFA\text{-}OOH + PUFA^{\cdot}. \quad (5)$$

One major consequence of lipid peroxidation which may have very important repercussions, is the change that has been shown to occur in the fluidity of the membrane lipid phase (Dobretsov *et al.* 1977; Slater, 1979; Vladimirov *et al.* 1980). This is known to have important effects on many of the major metabolic functions which are dependent on membrane structure (such as ion transport, maintenance of ionic gradients, etc., see Houslay & Stanley, 1982). Membrane fluidity can be studied in a number of ways, including ^{31}P nuclear magnetic resonance, polarized fluorescence and esr spin-labelling (Chapman & Hayward, 1985). In this latter technique a molecule (usually a fatty acid or cholesterol) labelled with a stable nitroxide radical is inserted into the membrane and the esr spectrum recorded. Analysis of the observed splittings of this radical can give information about the motion of this species and hence information about the fluidity of the membrane. Results obtained using fatty-acid spin-labels with the stable radical attached at different positions on the molecule have shown that there is an increase in the rigidity (decreased fluidity) of the membrane structure, with the effects most marked in the centre of the bilayer (Bruch & Thayer, 1983; Curtis *et al.* 1984). These changes are presumably due to the loss of PUFA which tend to make a membrane more fluid (Kusumi *et al.* 1986), or radical-induced cross linking of membrane components, or both.

Transition metal ions can have an exacerbating effect on lipid peroxidation, despite the fact that cells are, in general, well protected against the damaging effects of these ions. Complexation of iron (by transferrin, ferritin and haemosiderin; Bothwell *et al.* 1979) halts redox cycling of these metals, and these Fe 'pools' are normally compartmentalized well away from susceptible membrane sites. If, however, the cellular structure is disrupted in some way, such that this Fe becomes 'decompartmentalized' (Willson, 1977) or redox active, then rapid peroxidation may ensue by (a) the Fe-catalysing electron transfer to sites of radical production, (b) the Fe reacting with hydrogen peroxide to give $HO^{\cdot}$ radicals (reaction 6), or (c) by reacting with lipid hydroperoxides (PUFA-OOH, a product of lipid peroxidation) to yield more radicals (reaction 7), thus initiating a cascade process. The latter two processes have been extensively studied by esr spin-trapping (for review, see Davies, 1987). These reactions may account for the tissue damage observed in vivo in cases of Fe overload; for example, in kwashiorkor (Golden, 1987), the hind-limb paralysis observed in vitamin E-deficient piglets subjected to Fe–dextrose injections (Patterson *et al.* 1971), and in haemochromatosis (Peters *et al.* 1977).

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^- + HO^{\cdot} \quad (6)$$

$$Fe^{2+} + \text{PUFA-OOH} \longrightarrow Fe^{3+} + HO^- + \text{PUFA-O}^{\cdot} \quad (7)$$

The extent and rate of lipid peroxidation can be extensively modified by the presence of antioxidants. These compounds can be divided into two categories, preventative and chain-breaking (Burton *et al.* 1983), with the latter subdivided into water-soluble and lipid-soluble (see Table 3). Dietary constituents can have a profound effect on the antioxidant status of cells and tissues. Thus the trace metal selenium is necessary for the synthesis of glutathione peroxidase (*EC* 1.11.1.9) (Flohe *et al.* 1973), and copper, zinc, and manganese are essential for the activity of the various superoxide dismutases (*EC* 1.15.1.1) (Fridovich, 1982). The levels of chain-breaking antioxidants are obviously highly dependent on dietary levels of vitamin E, β-carotene, ascorbate (vitamin C) and cysteine (for glutathione synthesis). Metal chelators are used clinically to control excess levels of certain potentially damaging metal ions; for example penicillamine is used to complex and remove Cu in Wilson's disease, and the extremely potent Fe^{3+} chelator desferrioxamine (which has a binding constant for Fe^{3+} of 10^{31}) is used in the treatment of haemochromatosis.

Though the overall antioxidant capacity of human plasma has been evaluated (in percentage terms) as being α-tocopherol 5, ascorbate 15, urate 25 and protein thiols 50 (Wayner *et al.* 1985), it should be noted that synergistic interactions can occur between several of these compounds (Slater *et al.* 1987). As early as 1941 it was reported that vitamin C 'protected' vitamin E from destruction in foods (Golumbic & Mattill, 1941). Tappel (1968) has ascribed this protective effect to the repair of the vitamin E radical (produced by scavenging of a lipid peroxyl radical; reaction 8) by vitamin C (reaction 9). This process has been directly demonstrated by both pulse radiolysis and by esr (Packer *et al.* 1979; Davies *et al.* 1988). In the latter experiments the signal from the vitamin E phenoxyl radical (produced by reaction with $CCl_3O_2^{\cdot}$) was rapidly quenched by the addition of vitamin C, with the subsequent observation of the vitamin C radical.

$$\text{PUFA-OO}^{\cdot} + \text{vitamin E} \rightarrow \text{PUFA-OOH} + \text{vitamin E}^{\cdot}, \quad (8)$$

$$\text{vitamin E}^{\cdot} + \text{vitamin C} \rightarrow \text{vitamin E} + \text{vitamin C}^{\cdot}. \quad (9)$$

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It has been recently shown that a similar repair process can be carried out by various thiol compounds (at least when both compounds are free in solution; Davies *et al.* 1988). The esr signal from the vitamin E radical was removed by the addition of thiols, with this presumably being due to reaction 10. The resulting thiyl radicals ($RS^{\cdot}$) were detected by the use of the spin-trap DMPO which has previously been shown to be a good trap for these species (Davies *et al.* 1987). Several other free-radical scavengers may also act in a similar synergistic manner.

$$\text{vitamin E}^{\cdot} + \text{RSH} \longrightarrow \text{vitamin E} + \text{RS}^{\cdot}. \qquad (10)$$

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REFERENCES

Albano, E., Lott, K. A. K., Slater, T. F., Steir, A., Symons, M. C. R. & Tomasi, A. (1982). *Biochemical Journal* **204**, 593–603.

Beinert, H. (1972). In *Biological Applications of Electron Spin Resonance*, pp. 352–410 [H. M. Swartz, J. R. Bolton and D. C. Borg, editors]. New York: Wiley-Interscience.

Benedetto, C., Bocci, A., Dianzani, M. U., Ghiringhello, B., Slater, T. F., Tomasi, A. & Vannini, V. (1981). *Cancer Research* **41**, 2936–2942.

Borg, D. C. (1972). In *Biological Applications of Electron Spin Resonance*, pp. 265–350 [H. M. Swartz, J. R. Bolton and D. C. Borg, editors]. New York: Wiley-Interscience.

Bothwell, T. H., Charlton, R. W., Cook, J. D. & Finch, C. A. (1979). *Iron Metabolism in Man*, pp. 1–576. Oxford: Blackwell.

Bruch, R. C. & Thayer, W. S. (1983). *Biochimica et Biophysica Acta* **733**, 216–222.

Burton, G. W., Cheeseman, K. H., Doba, T., Ingold, K. U. & Slater, T. F. (1983). In *Biology of Vitamin E. Ciba Foundation Symposium* no. 101 (new series), pp. 4–14 [R. Porter and J. Wheelan, editors]. Amsterdam: Elsevier.

Bus, J. S. & Gibson, J. E. (1979). *Reviews in Biochemical Toxicology* **1**, 125–149.

Chapman, D. & Hayward, J. A. (1985). *Biochemical Journal* **228**, 281–295.

Cheeseman, K. H., Albano, E. F., Tomasi, A. & Slater, T. F. (1985). *Environmental Health Perspectives* **64**, 85–101.

Curtis, M. T., Gilfor, D. & Farber, J. L. (1984). *Archives of Biochemistry and Biophysics* **235**, 644–649.

Davies, M. J. (1987). *Chemistry and Physics of Lipids* **44**, 149–173.

Davies, M. J., Forni, L. G. & Shuter, S. L. (1987). *Chemico-biological Interactions* **61**, 177–188.

Davies, M. J., Forni, L. G. & Willson, R. L. (1988). *Biochemical Journal* **255**, 513–522.

Dianzani, M. U. & Ugazio, G. (1978). In *Biochemical Mechanisms of Liver Injury*, pp. 669–707 [T. F. Slater, editor]. London: Academic Press.

Dobretsov, G. E., Borschevskaya, T. A., Petrov, V. A. & Vladimirov, Y. U. (1977). *FEBS Letters* **84**, 125–128.

Dormandy, T. L. (1978). *Lancet* **i**, 647–650.

Finkelstein, E., Rosen, G. M. & Rauckman, E. J. (1980). *Archives in Biochemistry and Biophysics* **200**, 1–16.

Flohe, L., Gunzler, W. A. & Schock, H. H. (1973). *FEBS Letters* **32**, 132–134.

Floyd, R. A. (1980). In *Free Radicals in Biology*, vol. 4, pp. 187–208 [W. A. Pryor, editor]. New York: Academic Press.

Forni, L. G., Packer, J. E., Slater, T. F. & Willson, R. L. (1983). *Chemico-biological Interactions* **45**, 171–177.

Fridovich, I. (1982). In *Pathology of Oxygen*, pp. 1–17 [A. P. Autor, editor]. New York: Academic Press.

Golden, M. (1987). *Proceedings of the Nutrition Society* **46**, 53–68.

Golumbic, C. & Mattill, H. A. (1941). *Journal of the American Chemical Society* **63**, 1279–1280.

Halliwell, B. & Gutteridge, J. M. C. (1984). *Biochemical Journal* **219**, 1–14.

Halliwell, B. & Gutteridge, J. M. C. (1985). *Free Radicals in Biology and Medicine*. Oxford: Clarendon Press.

Houslay, M. D. & Stanley, K. K. (1982). *Dynamics of Biological Membranes*. New York: John Wiley.

Ingall, A., Lott, K. A. K., Slater, T. F., Finch, S. & Steir, A. (1978). *Biochemical Society Transactions* **6**, 962–964.

Janzen, E. G. (1980). In *Free Radicals in Biology,* vol. 4, pp. 115–154 [W. A. Pryor, editor]. New York: Academic Press.

Kalyanaraman, B. (1982). In *Reviews in Biochemical Toxicology,* vol. 4, pp. 73–139 [E. Hodgson, J. R. Bend and R. M. Philpot, editors]. New York: Elsevier Biomedical.

Kalyanaraman, B., Mottley, C. & Mason, R. P. (1983). *Journal of Biological Chemistry* **258,** 3855–3858.

Kusumi, A., Subczynski, W. K., Pasenkiewicz-Giericla, M., Hyde, J. S. & Merkle, H. (1986). *Biochimica et Biophysica Acta* **854,** 307–317.

Mason, R. P. (1982). In *Free Radicals in Biology,* vol. 5, pp. 161–222 [W. A. Pryor, editor]. New York: Academic Press.

Mason, R. P. (1984). In *Spin Labelling in Pharmacology,* pp. 87–129 [J. L. Holtzman, editor]. New York: Academic Press.

Mason, R. P. & Chignell, C. F. (1982). *Pharmacological Reviews* **33,** 189–211.

Packer, L. [editor] (1984). *Oxygen Radicals in Biological Systems, Methods in Enzymology,* vol. 105. Orlando: Academic Press.

Packer, J. E., Slater, T. F. & Willson, R. L. (1978). *Life Sciences* **23,** 2617–2620.

Packer, J. E., Slater, T. F. & Willson, R. L. (1979). *Nature,* **278,** 737–738.

Patterson, D. S. P., Allen, W. M., Berrett, S., Sweasey, D. & Dore, J. T. (1971). *Zentralblatt fur Veterinarmedizin* **A18,** 453–464.

Perkins, M. J. (1980). *Advances in Physical Organic Chemistry* **17,** 1–64.

Peters, T. J., Selden, C. & Seymour, C. A. (1977). In *Iron Metabolism. Ciba Foundation Symposium* no. 51 (new series), pp. 317–325 [R. Porter and D. Fitzsimons, editors]. Amsterdam: Elsevier, Excerpta Medica.

Poyer, J. L., McCay, P. B., Lai, E. K., Janzen, E. G. & Davis, E. R. (1980). *Biochemistry and Biophysics Research Communications* **94,** 1154–1160.

Pryor, W. A. (1966). *Free Radicals.* New York: McGraw-Hill Book Co.

Pryor, W. A. [editor] (1976–1986). *Free Radicals in Biology,* vol. 1–6. New York: Academic Press.

Recknagel, R. O. & Ghosal, A. K. (1966). *Laboratory Investigations* **15,** 132–147.

Rosen, G. M. & Finkelstein, E. (1985). *Advances in Free Radical Biology and Medicine* **1,** 345–375.

Slater, T. F. (1966). *Nature* **209,** 36–40.

Slater, T. F. (1972). *Free Radical Mechanisms in Tissue Injury.* London: Pion Ltd.

Slater, T. F. [editor] (1978). In *Biochemical Mechanisms of Liver Injury,* pp. 1–44. London: Academic Press.

Slater, T. F. (1979). In *Submolecular Biology and Cancer. Ciba Foundation Symposium* no. 67, pp. 301–328 [G. E. W. Wolstenholme, D. Fitzsimons and J. Whelan, editors]. Amsterdam: Elsevier, Excerpta Medica.

Slater, T. F. (1982). In *Free radicals, Lipid Peroxidation and Cancer,* pp. 243–270 [D.C. H. McBrien and T. F. Slater, editors]. London: Academic Press.

Slater, T. F. (1984). *Biochemical Journal* **222,** 1–15.

Slater, T. F., Cheeseman, K. H., Davies, M. J., Proudfoot, K. & Xin, W. (1987). *Proceedings of the Nutrition Society* **46,** 1–12.

Swartz, H. M., Bolton, J. R. & Borg, D. C. (1972). *Biological Applications of Electron Spin Resonance,* New York: Wiley-Interscience.

Tappel, A. L. (1968). *Geriatrics* **23,** 97–105.

Tomasi, A., Albano, E., Lott, K. A. K. & Slater, T. F. (1980). *FEBS Letters* **122,** 303–306.

Vladimirov, Y. A., Olenov, V. I., Suslova, T. B. & Cheremisina, Z. P. (1980). *Advances in Lipid Research* **17,** 173–249.

Wayner, D. D. M., Burton, G. W., Ingold, K. U. & Locke, S. (1985). *FEBS Letters* **187,** 33–37.

Willson, R. L. (1977). In *Iron Metabolism. Ciba Foundation Symposium* no. 51 (new series), pp. 331–354 [R. Porter and D. Fitzsimons, editors]. Amsterdam: Elsevier, Excerpta Medica.

Yamazaki, I. (1977). In *Free Radicals in Biology,* vol. 3, pp. 183–218 [W. A. Pryor, editor]. New York: Academic Press.

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