Inorganic Radicals of Relevance to Biological Systems [and Discussion]

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Inorganic radicals of relevance to biological systems

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There is little doubt that the most important inorganic radicals involved in biological systems are those which are intermediates in the oxygen–water redox cycle, i.e. $OH^{\cdot}$, O_2^- and $HO_2^{\cdot}$. Aspects of the structures and reactivities of these radicals are considered, together with methods of detection. In particular, the use of e.s.r. spectroscopy is outlined, including rapid-freeze and spin-trapping techniques. Attention is called to comparisons and contrasts between these radicals and corresponding sulphur-centred radicals, although these are not strictly 'inorganic'. The oxygen-centred radicals are usually generated *in vivo* by redox reactions, but they are also of importance in radiolytic processes because they are formed from water. Other radicals formed in this way whose structures and reactivities are considered include solvated electrons and hydrogen atoms.

1. Background

Definitions are always difficult and are best avoided. Nevertheless, a working definition for the present task is that radicals are species containing one, or possibly more than one, unpaired electron; they display a tendency to form bonds to other species (molecules, ions or radicals) such that their unpaired electrons become paired. Examples of typical reactions for a radical $X^{\cdot}$ are dimerization, (1), hydrogen-atom abstraction, (2), and radical addition, (3). All but reaction (1) result in the generation of new radicals.

$$2X^{\cdot} \rightarrow X\text{—}X, \tag{1}$$

$$X^{\cdot} + RH \rightarrow X\text{—}H + R^{\cdot}, \tag{2}$$

$$X^{\cdot} + A\text{—}B \rightarrow X\text{—}A\text{—}B^{\cdot}. \tag{3}$$

Radicals are also frequently good electron donors (especially radical anions) or good electron acceptors (especially radical cations), but these are not properties that are in any sense unique to radicals. Many transition metal complexes contain unpaired d-electrons but, in general, these do not exhibit reactions of type (1)–(3) and hence, with some exceptions, do not qualify for the description 'radical'.

A few inorganic radicals are stable with respect to dimerization. These include $NO^{\cdot}$, which forms a weakly bound dimer at low temperatures; O_2^-, which probably does not dimerize (but can form a weak complex with O_2 to give O_4^-; Lindsay *et al.* 1983); $^{\cdot}NO_2$, which forms N_2O_4 reversibly at room temperature; O_3^-, the oxonide ion which has no well defined dimer; $^{\cdot}NF_2$, which is formed reversibly from N_2F_4; $^{\cdot}SO_2^-$, formed in solution from dithionite ions; $S_2O_4^{2-}$, and $^{\cdot}ClO_2$, which shows no tendency to dimerize. All but $^{\cdot}NO_2$ are π^*-radicals in that their SOMOs (semi-occupied molecular orbitals) are π^* in nature and are strongly delocalized over the molecular frames. Other radicals of interest in the present context will dimerize irreversibly if no other mode of reaction presents itself. Thus, for example, $OH^{\cdot}$ radicals give H_2O_2. In contrast, $HO_2^{\cdot}$ radicals, which in principle can give HO_4H dimers, undergo a preferable disproportionation to give H_2O+O_2.

After the beautifully unifying concepts of nucleophilic and electrophilic reaction mechanisms were developed in the thirties (Ingold 1953), there was considerable resistance to the idea that homolytic mechanisms might apply to reactions in solvents, especially in aqueous solutions. There was no easy way of establishing the involvement of radicals until M. G. Evans and his coworkers, who included Polanyi, Baxendale and Uri, showed that free-radical polymerization was a sensitive method of trapping radicals, greatly magnifying their effect via long chain processes (Baxendale *et al.* 1946). For example, if a dilute aqueous solution of acrylonitrile is treated with a drop of a radical-generating solution, the whole solution becomes filled with precipitated polymer in a most dramatic fashion.

In particular, these studies strongly supported the concept that induced oxidations from Fenton's reagent and related systems proceeded via the formation of $OH^{\cdot}$ radicals (4).

$$Fe^{2+}+H_2O_2 \rightarrow Fe^{3+}OH^{-}+OH^{\cdot}. \tag{4}$$

In the presence of most organic compounds, the highly reactive $OH^{\cdot}$ radicals react to give induced oxidations rather than reacting further with the iron complexes or with H_2O_2.

My own initiation into this area of chemistry arose when I noticed that permanganate ions reacted smoothly in strongly alkaline aqueous solutions to give manganate and oxygen, and that this system could induce the oxidation of organic compounds (R—H) which did not react with MnO_4^- directly (Symons 1953*a*, *b*). I suggested that reactions (5) and (6) were responsible:

$$MnO_4^{-}+OH^{-} \rightleftharpoons MnO_4^{2-}+{}^{\cdot}OH, \tag{5}$$

$${}^{\cdot}OH+RH \rightarrow H_2O+R^{\cdot}. \tag{6}$$

Subsequent studies seemed to give strong support to the idea (Symons 1953*a*, *b*, 1954; Lott & Symons 1960; Carrington & Symons 1963). This reaction hinges on the fact that the electron-donating ability of OH^- increases steadily as its solvation by water is reduced. Thus the reaction is only of use in strongly alkaline media and has not been used very widely.

Because of the prevalence of transition metals, particular iron, in biological systems, it is inevitable that reactions with H_2O_2 will be potential sources of $OH^{\cdot}$ radicals and of O_2^-–$HO_2^{\cdot}$ radicals, while oxygen is always a source of O_2^-–$HO_2^{\cdot}$ radicals. (It is necessary to consider both O_2^- and $HO_2^{\cdot}$ in this context, because the pK_a of $HO_2^{\cdot}$ is 4.9, so that formation of O_2^- is always accompanied by some $HO_2^{\cdot}$. This is important because of the very different reactivities of these species.) Sometimes the system is designed to produce these radicals in abundance, because of their high reactivities, but otherwise it is necessary to keep their concentrations low, and Nature has developed many efficient ways for accomplishing this task.

My aim is to make some general comments about these inorganic radicals. Three aspects are considered: electronic structures; reactivities; and methods of detection. These topics are closely interlinked, because reactivities stem largely from the nature of the SOMOs of the radicals and of the neighbouring filled and empty levels. It is these SOMOs that are so well probed by e.s.r. spectroscopy.

2. Detection of radicals

At this stage I consider, briefly, various methods of detection of radicals before considering their structures, because I can then use spectroscopic evidence in support of my statements.

(*a*) *Chemical methods*

These are legion, and include the induced oxidation and polymerization tests mentioned above. Some of the best are enzymatic, such as the use of superoxide dismutase to remove O_2^- radicals selectively. The spin-trapping technique outlined in §2*d* is an excellent example of a chemical test coupled with e.s.r. spectroscopy. My concern in this review is, however, not with chemical methods and their many limitations, but with spectroscopic techniques.

(*b*) *Optical methods*

Ultraviolet and infrared absorption, ultraviolet emission and Raman or resonance Raman scattering techniques have all been used effectively in the study of radicals. Of these, infrared and normal Raman techniques are relatively insensitive, so that high concentrations of radicals are required. Resonance Raman is far more sensitive, but it can at present only be used for radicals having well defined, low-lying allowed electronic transitions. The use of tunable u.v. lasers promises to open up this technique, but the problem may then be that systems of interest may absorb the incident light too strongly to permit the extraction of useful information. Ultraviolet spectroscopy has proven to be the most powerful of these techniques, but firm identification of the absorbing species is sometimes difficult, because the information relates to the difference between ground and excited states and is only a good 'fingerprint' if prior knowledge of the spectrum is available. Furthermore, many radicals of interest have poorly defined u.v. spectra, or are transparent in the normal range.

(*c*) *Electron spin resonance*

This technique is uniquely applicable to radicals and to paramagnetic transition metal complexes because it detects transitions between electron spin levels, and these are confined to systems containing unpaired electrons. There are many books and reviews which outline the theory (Wertz & Bolton 1972; Atherton 1973) and chemical applications (Symons 1978) of this technique, and one which is solely concerned with inorganic radicals (Atkins & Symons 1967). I confine my attention here to a brief overview of the method, to clarify later comments. This is most readily accomplished by examining figures 1–7 and by reference to the closely related and far more widely known technique of n.m.r. spectroscopy.

Figure 1 shows the act of absorption, namely the electron spin-flip transition induced by microwave radiation where the degeneracy of the $S = \pm\frac{1}{2}$ levels has been lifted by a static magnetic field (B_z). Instrumentation is very similar to that for n.m.r. spectroscopy, except that it is not possible to sweep the microwave field, so it is normal practice to sweep the applied magnetic field. The effect of this on the energy levels is shown in figure 2. This shows that the rate of divergence of the electron energy levels, which is governed by the g value ($h\nu = g\mu_\beta B$, when ν is the microwave frequency and B the applied magnetic field), can vary. If there is no orbital motion of the unpaired electron, $g = 2.0023$. This can be thought of as the 'free-spin' value and taken as an effective zero. Orbital motion leading to an orbital magnetic moment occurs when the SOMO is close in energy to empty (virtual) or filled orbitals with which it

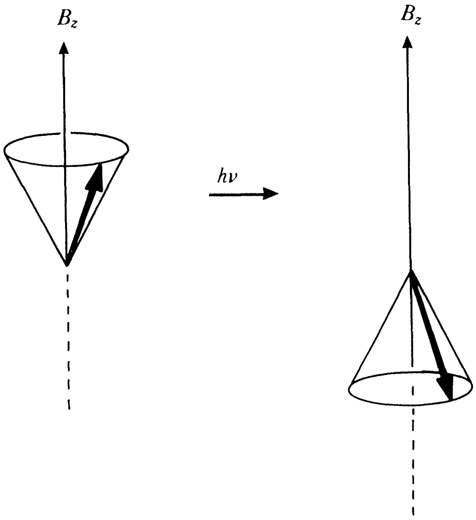


FIGURE 1. The e.s.r. transition induced by the microwave field ($h\nu$). The electron is pictured as a small magnet (arrow) precessing about the direction of the applied magnetic field B_z.

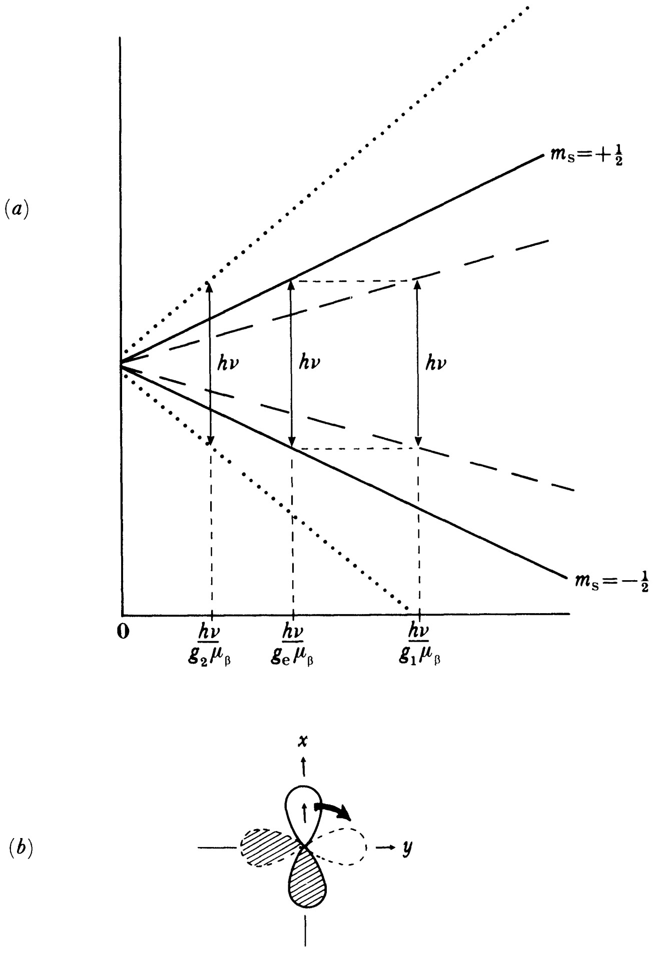


FIGURE 2. (*a*) The effect of an applied static magnetic field (B) on the $M_S = \pm\frac{1}{2}$ levels of an unpaired electron. These are degenerate at zero field. The thick lines represent the spin-only behaviour, the transition ($h\nu$) occurring at a field corresponding to $g_e = 2.0023$. The broken lines are for a radical having a low-lying vacant excited state, giving $g_1 < 2.0023$, and the dotted lines are for a radical having a neighbouring filled level, giving $g_2 > 2.0023$. (Taken from Symons 1978.) (*b*) Movement of the unpaired electron from one p-orbital (p_x) into another (p_y) induced by a magnetic field (B_z) gives rise to orbital motion and hence a shift in the g value.

can be magnetically coupled. As indicated in figure 2, Δg is negative (shift to high field) if the coupled level is empty and positive if it is filled.

For most organic radicals, shifts in g are small and are not of major diagnostic value. However, for many transition metal complexes, and for several of the oxygen- and sulphur-centred radicals discussed herein, Δg can be large, and controls the form of the e.s.r. spectra.

The other major factor controlling the form of the e.s.r. spectrum is electron–nuclear hyperfine coupling, which can be compared with nuclear–nuclear spin-coupling in n.m.r. spectroscopy.

This leads to a splitting of the $M_S = \pm\frac{1}{2}$ states as indicated in figure 3, the resulting pattern of lines being characteristic of the type, and number of coupled nuclei. Some examples are shown in figures 4–6. In particular, for the much studied nitroxide radicals, $R_2\dot{N}O$, mentioned below, coupling to the ^{14}N nucleus results in three equally intense lines (figure 5*a*) because $I = 1$. Thus, $\frac{1}{3}$ of the radicals have $M_I = +1$, $\frac{1}{3}$ have $M_I = 0$ and $\frac{1}{3}$ have $M_I = -1$. In favourable cases, hyperfine coupling to other nuclei, including ^1H and ^{13}C is also observed in the spectra of $R_2\dot{N}O$ radicals (figures 7 and 8).

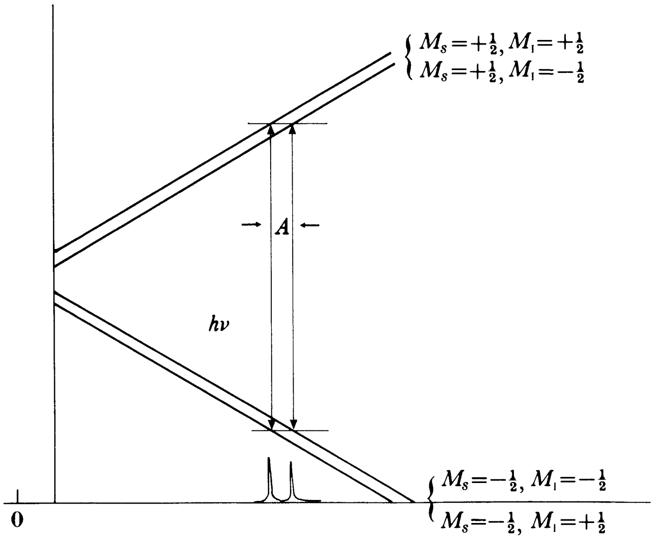


FIGURE 3. Divergence with field of the $M_S = \pm\frac{1}{2}$ levels in the presence of a single nucleus having $I = \frac{1}{2}$, in the high-field approximation. Note the two allowed transitions involve no change in M_I.

As with n.m.r. spectra, variation in linewidths can be used to extract kinetic information about the radicals, and changes in the hyperfine parameters can be used to estimate the degree of solvation and hence the environment of the radicals. It is important to note that both g and A are, in general, anisotropic, having x, y and z components. Liquid-phase spectra are averages, and give only the isotropic parts of these tensors, and the rate of averaging may make important contributions to the linewidths.

In some cases, the effects of anisotropy and other factors are so great that e.s.r. signals are too broad to detect in the liquid phase. One approach is then to freeze the solution and measure the solid-state e.s.r. spectrum. This is a less sensitive procedure because the resulting 'powder' spectra may cover a wide field range, as illustrated in figure 9 for a solution containing O_2^-.

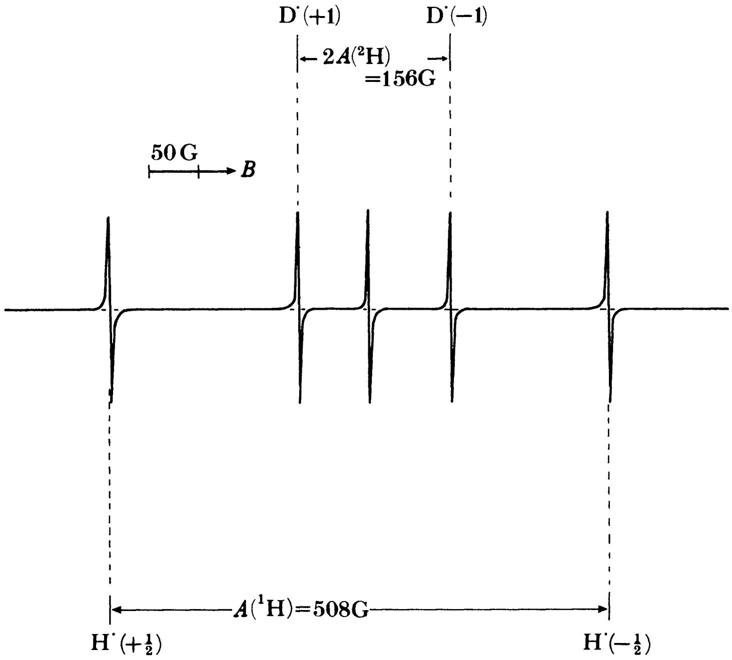


FIGURE 4. First derivative e.s.r. spectrum showing features for trapped hydrogen and deuterium atoms.

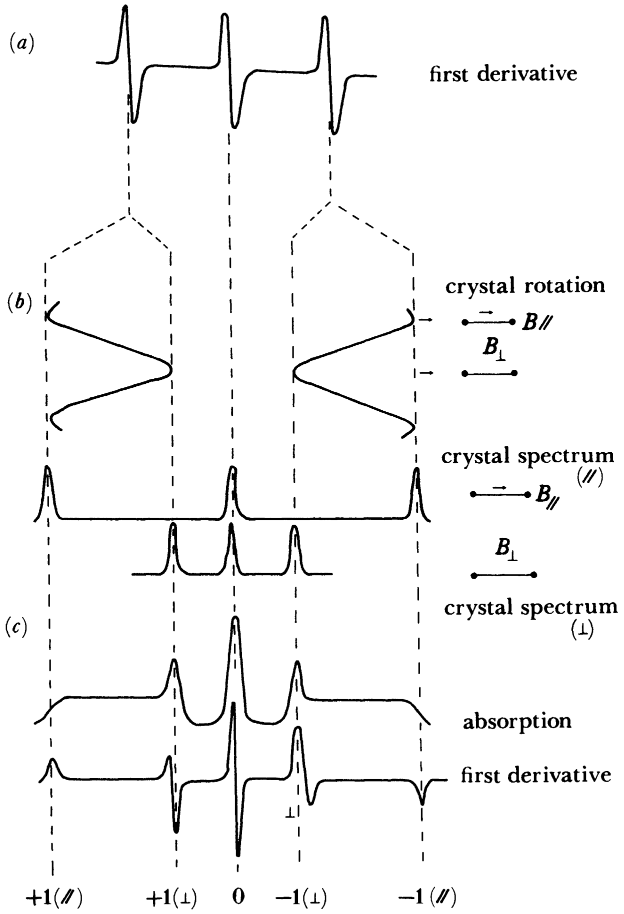


FIGURE 5. This figure illustrates the connection between a typical solution spectrum for a radical with a single coupled nucleus with $I = 1$, (*a*), and the changes on rotation observed for a single crystal. (*b*) The rotation is selected such that the parallel and perpendicular orientations are traversed. An isotropic g value has been assumed. The corresponding powder spectrum in absorption and as the first derivative is given in (*c*).

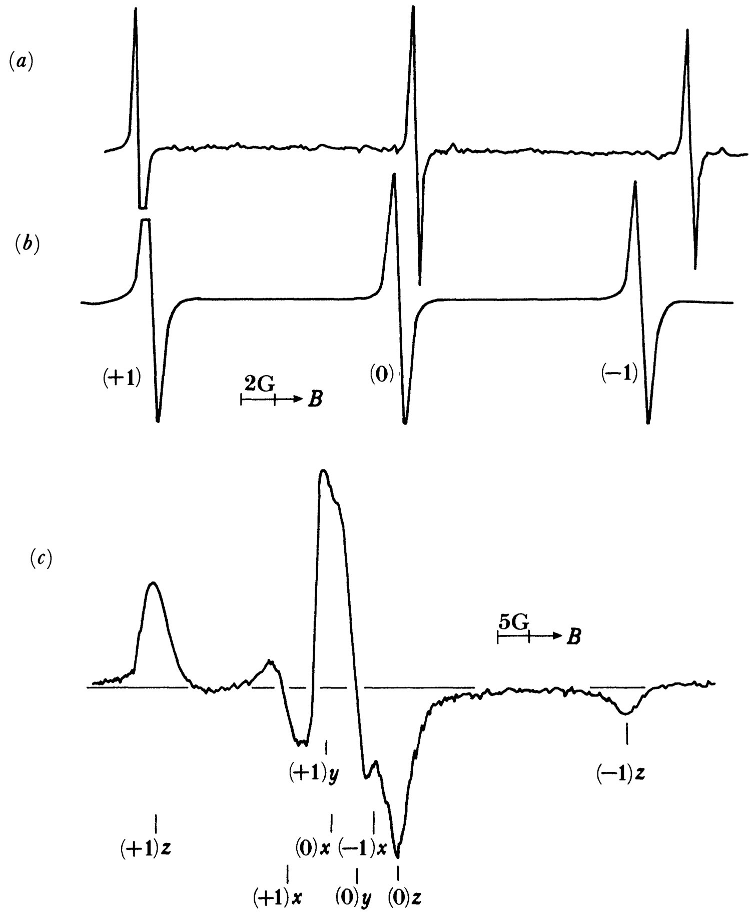


FIGURE 6. First derivative X-band e.s.r. spectra for $(Me_3C)_2NO^{\cdot}$ radicals (*a*) in water at 20 °C; (*b*) in dodecane at 20 °C and (*c*) in CD_3OD at 77 K. In (*c*) $(CD_3)_3CNO^{\cdot}$ radicals were used to give better definition.

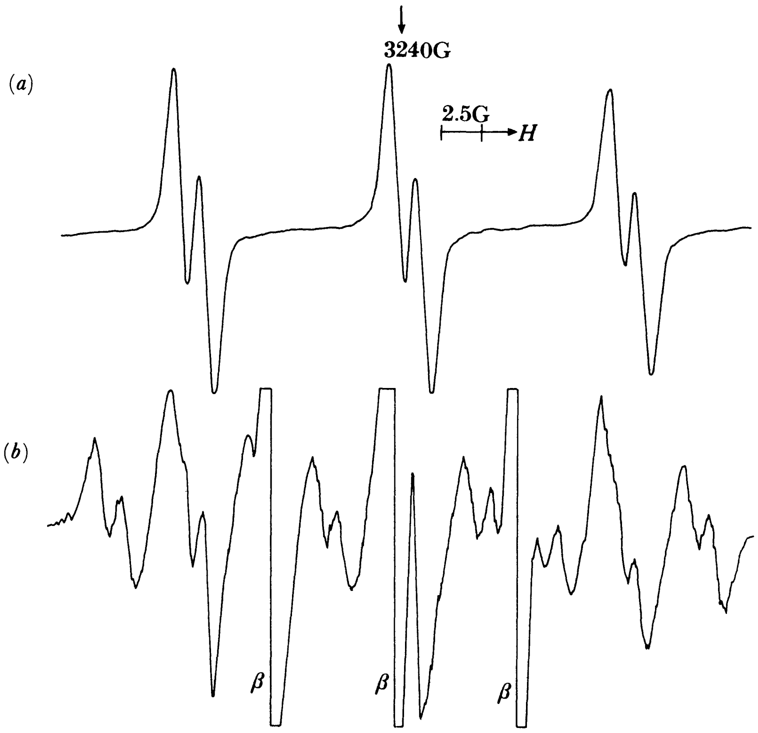


FIGURE 7. First derivative X-band e.s.r. spectra for (*a*) PBN and $^{\cdot}[^{12}C]Cl_3$; (*b*) PBN + $^{\cdot}[^{13}C]Cl_3$.

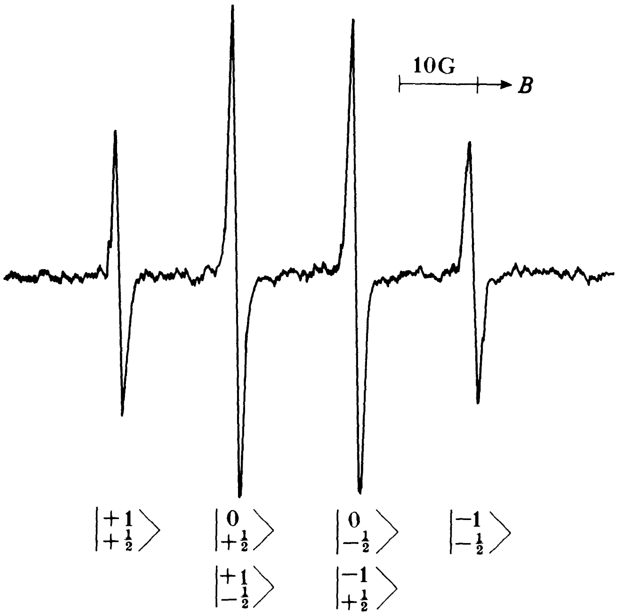


FIGURE 8. First derivative X-band e.s.r. spectrum assigned to the OH˙ radical adduct of DMPO. Note that $A(^{1}\text{H}) \approx A(^{14}\text{N})$ so that the spectrum is a quartet.

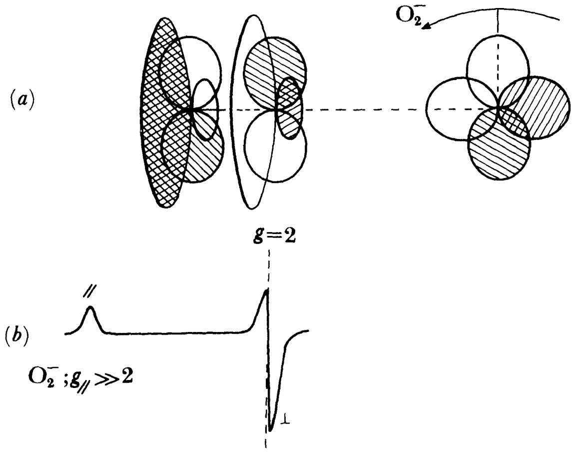
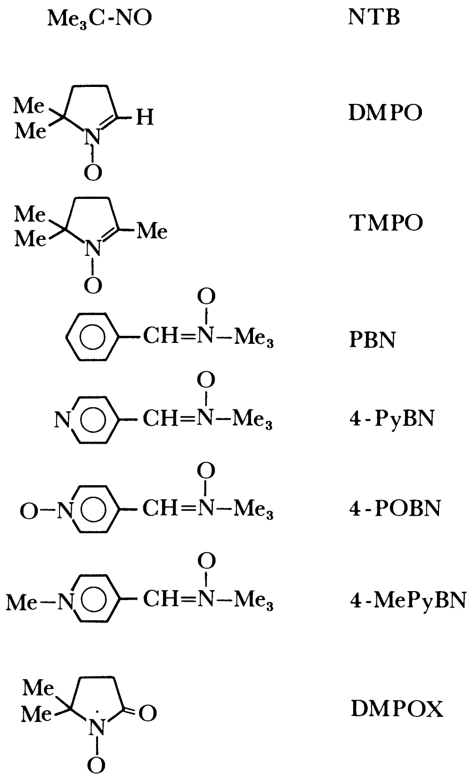


FIGURE 9. (a) The π-orbitals for O_2^- and (b) the resulting e.s.r. spectrum.

Also, for aqueous systems, phase separation giving ice as the dominant phase almost always occurs, and this can lead to spin-exchange effects which often modify the e.s.r. spectra.

(d) *Spin trapping*

In these cases, and in situations for which the stationary concentration of reactive radicals is below the level of the instrument sensitivity (for narrow-line species radicals at concentrations of *ca.* 10^{-7} mol^{-1} dm^{-3} detected), it is convenient to use various spin traps, as illustrated in scheme 1. The essential feature of this procedure (Janzen & Blackburn 1968; Chalfont *et al.* 1968) are that additions to the trap must be rapid, and the resulting nitroxide radical must



SCHEME 1. Examples of commonly used spin traps.

be far more stable than the parent radical, so that relatively high concentrations can accumulate.

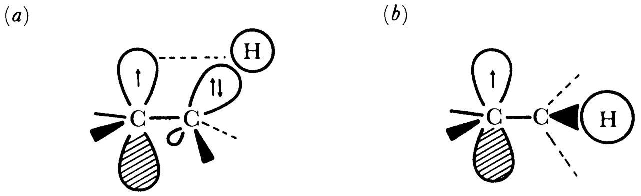
Although this is a good method for proving the participation of radicals, identification and attempts to quantify yields are fraught with difficulties (Janzen 1971). Some of these are briefly indicated below.

Identification frequently rests upon a fingerprinting process, in which an established spectrum is used as a gauge. This presupposes that all radical adducts of a given trap have unique spectra, which may sometimes be a dangerous concept. The following considerations are pertinent.

(i) $A(^{14}N)$ varies slightly with the nature of $R^{\cdot}$. This is especially the case for RNO traps.

(ii) For nitrone traps, the unique β-proton gives a variable coupling. This is determined by the degree of overlap between the π^*-orbital containing the unpaired electron and the C—H σ-orbital (θ in figure 10; the proton coupling is proportional to $\cos^2\theta$). The nature of R, including its bulk, will govern the equilibrium value of θ. Thus $A(^1H)$ is a strongly variable parameter and hence a useful tool for identification.

(iii) The most convincing datum, if it can be seen, is direct hyperfine splitting from one or more nuclei of the added radical, $R^{\cdot}$. A nice example of this is the doublet splitting from ^{13}C for $^{\cdot}[^{13}C]Cl_3$ radicals, shown in figure 7 (Symons *et al.* 1982). This provides unambiguous evidence that $^{\cdot}CCl_3$ radicals were being trapped. Hyperfine coupling to protons in $R^{\cdot}$ is usually small, and lost in the linewidths. However, such coupling can sometimes be detected by using resolution enhancement techniques, which comprise computer processing of the data, or better, by using ENDOR (electron–nuclear double resonance) methods.



maximum σ-π overlap for C-H bond: $\theta = 0°$

zero σ-π overlap for C-H bond: $\theta = 90°$

Figure 10. Overlap (σ–π or hyperconjugative) for a C—H bond in a radical $R_2\dot{C}—CHR'_2$ showing how this depends upon θ, the angle between this bond and the radical plane.

Thus, ideally, oxygen-centred radicals should be studied by using ^{17}O enrichment. This nucleus has $I = \frac{5}{2}$ and hence splitting of each component into sextets would be observed. I am not aware of any such studies for the oxygen-centred radicals discussed herein.

I now turn to a consideration of the structures and reactivities of various oxygen-centred radicals, because these are by far the most important inorganic radicals involved in biological systems.

3. Dioxygen derivatives

(a) *Dioxygen*

Dioxygen has a ground-state triplet level, the two unpaired electrons being accommodated, formally, in the degenerate pair of antibonding π-orbitals, $\pi_x^*(\uparrow), \pi_y^*(\uparrow)$. It is noteworthy that, because of strong coupling to rotational levels, the e.s.r. spectrum for O_2 as a low-pressure gas comprises sets of many narrow lines spread over a wide field range. Unfortunately, these are so extensively broadened for O_2 in solution that no resonance is detectable. However, oxygen is an important source of internal fluctuating magnetic fields, which may broaden the e.s.r. features of other radicals.

It is important to note that there are several low-lying excited states for dioxygen, probably the most important being the $^1\Delta$ state in which the electrons are paired in one of the π*-orbitals, leaving the other vacant. Again, the $(\pi_x^*)^2$, $(\pi_y^*)^0$ description is not strictly correct for the gas-phase molecule, but I suggest that it may be suitable for $^1\Delta O_2$ in aqueous solution. This possibly significant suggestion stems from our knowledge of the asymmetric solvation of O_2^-, in which hydrogen bonding is directed towards the filled π*-orbital only (cf. figure 11). I think some elements of this solvation would also occur for aqueous solutions of the $^1\Delta$ form of dioxygen. This would serve to distinguish between π_x and π_y. It might also have a measurable effect on the emission spectrum for $^1\Delta$ as it falls to the ground state. Such an effect could be important because this spectrum is used for identification of singlet dioxygen (Kanofsky 1983, 1984).

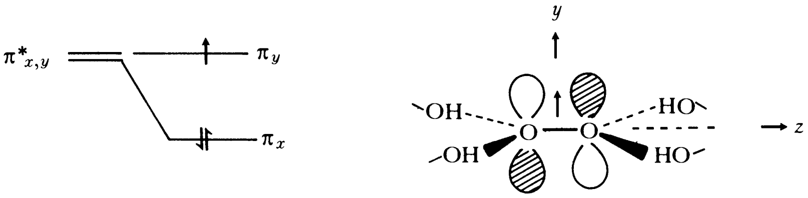


Figure 11. Effect of solvation (hydrogen bonding) on the π_x^*- and π_y^*-orbitals for the superoxide ion. Bonding is largely confined to the x–z plane.

The reactivity of $^1\Delta O_2$ is clearly different from that for the ground, $^3\Sigma$, state. The latter reacts as a bi-radical, and it is a good radical scavenger, whereas the former can react both as a nucleophile and as an electrophile. Because O_2 is thought to be produced in its $^1\Delta$ form in certain reactions, and because the spin-flip transition to the ground state is only weakly allowed, this reactivity difference may be important.

(*b*) *The superoxide ion*

Oxygen has a high electron affinity, the excess electrons being accommodated in the antibonding $\pi_{x,y}$ level. Again there is strong spin–orbit coupling, but for O_2^- in solution or in its salts the degeneracy of the π_x^*- and π_y^*-orbitals is lifted by hydrogen bonding or crystal-field effects, as indicated in figure 11. This has the effect of largely quenching orbital motion but, nevertheless, when a magnetic field, B, lies along the molecular axis (z) orbital motion is encouraged and increases linearly with field, thus increasing the rate of divergence of the spin-doublet levels (g_z) (figure 2). This facile circulation cannot occur for B_x or B_y so, in first order, g_x and g_y remain close to the normal free-spin value.

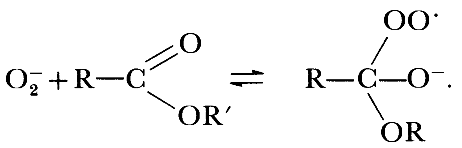
I stress that the magnitude of the down-field shift for g_z is not so much a property of the O_2^- radical as of the strength of the electric field governing Δ. This must be precise if a well defined z feature is to be observed in the solid-state e.s.r. spectrum. In the liquid phase there are two uncertainties. One is that the signal is located at the weighted average of the g values, and the other is that the formal g_z value will itself fluctuate as the solvation is modulated by the normal motionally induced changes in solvation. The result is a line too broad for detection by e.s.r.

Some of these effects are nicely illustrated by our e.s.r. results for the growth of solvation for O_2^- radicals generated by radiation-induced electron addition at 4 K (Eastland & Symons 1977; Symons & Stephenson 1981). Initially, the oxygen molecules in solvents such as ethanol exist in solvent cages, being surrounded by ROH molecules hydrogen bonded to themselves, not to oxygen. When these glassy solutions are exposed to ionizing radiation at 4 K, electron addition occurs (as shown by a decreased yield of trapped electrons), but no e.s.r. signals characteristic of O_2^- are observable. As the temperature is raised, a broad, very low-field g_z feature appears, which narrows and moves to higher fields in discrete stages as the temperature is raised. At *ca.* 90 K this feature has become identical with that observed from normal frozen solutions of NaO_2 in ethanol. We conclude that we are observing the progressive addition of EtOH molecules to O_2^- by hydrogen bonding, the changes indicating that at equilibrium there are four such bonds. To explain the consequent splitting of the π_x^*- and π_y^*-orbitals, this solvation must be confined to a planar arrangement. These arguments have been strongly supported by recent electron spin-echo studies (Narayama & Kevan 1980).

The reactivity of the superoxide ion is relatively low. It forms stable salts, and shows little tendency to dimerize. Its reactivity is twofold: it is a one-electron donor, and it is a base (or nucleophile). Its electron-donating power is a function of solvation: the anion is strongly stabilized in aqueous solution, and hence will have a lower electron-donating power than in an aprotic environment.

Protonation occurs in water with a pK_a of *ca.* 4.9. This means that there will still be significant protonation in neutral solutions, and because the reactivity of $HO_2^{\cdot}$ is far greater than that of O_2^- in certain respects, some reactions exhibited by aqueous superoxide solutions may well be due to $HO_2^{\cdot}$.

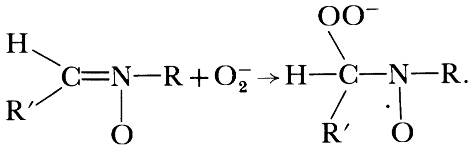
The superoxide ion also acts as a nucleophile in some of its reactions, especially towards carbonyl groups, as in its reactions with esters, (7) (Galliani & Rindone 1981).



$$O_2^- + R{-}C({=}O){-}OR' \rightleftharpoons R{-}C(OO^{\cdot})(OR){-}O^- . \qquad (7)$$

Subsequent reactions are not yet well established (Johlman *et al.* 1983), but such nucleophilic addition has received strong support from the observation of a radical having g values close to those for normal $ROO^{\cdot}$ radicals (see below) when superoxide ions react with dimethylformamide (Green *et al.* 1979; Symons *et al.* 1980). It seems that subsequent reactions are less facile in anhydrous solutions: addition of water leads to re-formation of solvated O_2^- ions, rather than to break-down products as with the ester adducts.

Note that in this addition the e.s.r. results support the concept that O_2^- adds to the $C{=}O$ group via electron-pair donation; as a nucleophile not a radical. So the SOMO remains a π^* oxygen orbital just as it does on protonation. In contrast, addition of O_2^- to the $C{=}N$ double bond of a nitrone involves the SOMO of the anion, so the negative charge is retained by oxygen and the SOMO becomes the normal $N{-}O$ π^*-orbital (8).



$$HR'C{=}N(O){-}R + O_2^- \rightarrow H{-}C(OO^-)(R'){-}N(\dot{O}){-}R. \qquad (8)$$

This dichotomy in reactivity appears to be well defined. However, it may not always be clear which mechanistic role is involved. Thus, for example, in its addition to $>C{=}O$ to give $>C(OO^{\cdot})(O^-)$ adducts, the obvious route involves direct nucleophilic attack (7). However, the alternative radical mode, comparable with (8), gives $>C(OO^-)(O^{\cdot})$, but this would surely undergo internal electron-transfer to give the former species very rapidly. Thus the distinction is somewhat esoteric. However, for bimolecular displacements, such as with alkyl halides, the distinction should be clear because as a nucleophile the products would be $ROO^{\cdot}$ radicals and halide ions, whereas as a radical they would be RO_2^- anions and halogen atoms. The former would be relatively more favoured for chlorides, but the latter would be relatively more favoured for iodides.

Presumably the radical $O^{-\cdot}$ should show a similar dichotomy in its reactivity. Both mechanisms lead to the same product for addition to $>C{=}O$ units, but the products would be distinct for displacement reactions with alkyl halides.

(*c*) $HO_2^{\cdot}$ *and* $RO_2^{\cdot}$ *radicals*

Apart from the proton splitting for the former, these two radicals have very similar e.s.r. spectra (table 1). The $HO_2^{\cdot}$ radical has never been detected in fluid solution, but is readily

TABLE 1. TYPICAL E.S.R. PARAMETERS FOR VARIOUS INORGANIC RADICALS

radical	matrix	g values				nucleus	hyperfine coupling constants/G[a]			
		g_x	g_y	g_z	g_{av}		A_x	A_y	A_z	A_{iso}
$O^{-\cdot}$	D_2O/NaOD[b]	2.070	2.002	2.002	2.025	—	—	—	—	—
$OH^{\cdot}$	H_2O[c]	2.059	2.009	2.0027	—	^1H	0	45	28.5	24.5
O_2^-	MeOH + H_2O[d]	2.078	2.000	2.000	2.026	—	—	—	—	—
$HO_2^{\cdot}$	$H_2O + H_2O_2$[e]	2.0353	2.0086	2.0042	2.0160	^1H	13.8	3.6	15.7	11.0
$O_3^{-\cdot}$	$KClO_3$[f]	2.0025	2.0174	2.0113	2.0104	—	—	—	—	—
$Cl_2^{-\cdot}$	H_2O[g]	2.000	2.040	2.040	2.027	^{35}Cl	102	10	10	40.8
$ClOH^{-\cdot}$	H_2O[gh]	2.004	2.017	2.017	2.013	^{35}Cl	59	−16	−16	9
$ClOO^{\cdot}$	$KClO_3$[f]	1.9983	2.0017	2.0130	2.0043	^{35}Cl	5.3	7.2	14.9	9.1
$ClO^{\cdot}$	CO_2[i]	1.889	1.899	2.66	2.149	^{35}Cl	30	*ca.* 0	*ca.* 0	*ca.* 10
$^{\cdot}NO_2$	H_2O[j]	2.0066	1.9920	2.0022	2.0003	^{14}N	50.6	49.84	70.21	56.88
$^{\cdot}SO_2^-$	H_2O[kl]	—	—	—	2.0057	^{33}S	—	—	—	14.2
		2.0026	2.0026	2.0026	2.0026	^{33}S	153	115.5	115.5	128
$^{\cdot}SO_3^-$	H_2O[m]	—	—	—	2.0028					

[a] 1 G = 10^{-4} T.
[b] Blandamer *et al.* 1964.
[c] Brivati *et al.* 1967.
[d] Symons & Stephenson 1981.
[e] Wyard *et al.* 1968.
[f] Eachus *et al.* 1968.
[g] Catton & Symons 1969.
[h] Ginns & Symons 1972.
[i] Trainer *et al.* 1983.
[j] Atkins *et al.* 1962.
[k] Atkins *et al.* 1964.
[l] Chantry *et al.* 1962.
[m] Ozawa & Kwan 1983.

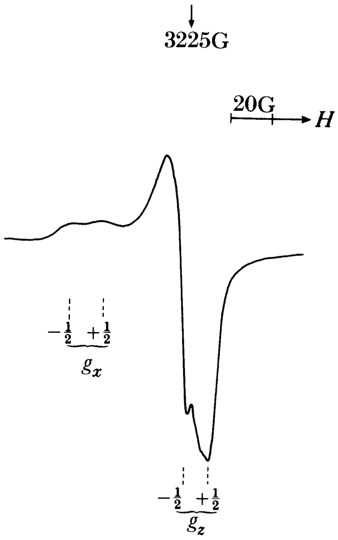


FIGURE 12. First derivative X-band e.s.r. spectrum for $H_2O + H_2O_2$ after exposure to ^{60}CO γ-rays at 77 K and on cooling to *ca.* 150 K, showing features assigned to $HO_2^{\cdot}$ radicals.

characterized in glassy or crystalline media (figure 12) (Catton & Symons 1969*b*). However, $RO_2^{\cdot}$ radicals such as $Me_3COO^{\cdot}$ are readily detectable in fluid solution although the spectra are always broad unresolved singlets (Ingold 1969; Kalyanaraman *et al.* 1983). Two factors contribute to this width: at low temperatures, uncertainty in the averaging of the *g* anisotropy is the major factor, while at higher temperatures incipient rotational quantization, usually called 'spin rotation', causes an alternative broadening. Thus there are optimum temperatures at which fairly narrow lines can be produced. For $HO_2^{\cdot}$ in water, strong hydrogen bonding will inhibit spin-rotation broadening, but fluctuations in solvation will undoubtedly contribute to line-broadening. However, the g values are now primarily determined by the structure of the radicals, which are markedly bent. Thus one of the degenerate π-orbitals of O_2^- is involved in covalent bonding to H or R, the SOMO being strongly confined to the other π^*-orbital. Even so, coupling can still occur, as evidenced by the well defined shift in g_z (*ca.* 2.034). Unfortunately, hyperfine coupling to protons in the alkyl units is not resolved, so it is not possible to identify specific $RO_2^{\cdot}$ radicals, though, in principle, this might be achieved by using ENDOR or electron spin–echo techniques.

As stressed above, $HO_2^{\cdot}$ radicals are of importance in solutions containing O_2^- ions. Their reactivity, apart from being weak acids, is centred largely on the unpaired electron. They are less reactive than $OH^{\cdot}$ radicals, because the SOMO is delocalized, but they do dimerize reversibly to give unstable tetroxides, RO_4R. They can add to double bonds, as, for example, in their addition to the C=N bonds of nitrones, their reactivity being far greater in this respect than that of O_2^-. Thus it may well be $HO_2^{\cdot}$ that is spin trapped by nitrones rather than O_2^- in neutral aqueous solutions.

A major switch in redox behaviour occurs when O_2^- is protonated, because $HO_2^{\cdot}$ is a very poor electron donor, but a good electron acceptor. The resulting HO_2^- (or RO_2^-) anions are quite strongly basic, and protonate reversibly to give H_2O_2 or ROOH molecules. This again greatly modifies the redox properties, because HO_2^- is a good electron donor, while H_2O_2 is a good acceptor (9).

$$H_2O_2 + e^- \rightarrow OH^{\cdot} + OH^-. \tag{9}$$

This alternation in electron-donating and accepting behaviour on proton gain or loss is, of course, of vital importance in the chain of events connecting O_2 and H_2O.

These radicals ($HO_2^{\cdot}$ and $RO_2^{\cdot}$) are also able to abstract hydrogen from C—H bonds, particularly if these are activated. A very important example is step (10) in the reaction chain that leads to efficient lipid peroxidation:

$$R^{\cdot} + O_2 \rightleftharpoons RO_2^{\cdot}, \tag{10}$$

$$RO_2^{\cdot} + \text{—CH=CH—CH}_2\text{—} \rightarrow RO_2H + \text{—}\overline{\dot{\text{C}}\text{H—CH—}\dot{\text{C}}\text{H}}\text{—}. \tag{11}$$

Reaction (10), which occurs with high efficiency, is an important example of the reactivity of dioxygen, and is of major significance because it converts a relatively stable radical into one with greater reactivity.

4. The hydroxyl radical

In many ways, this is the most important of the oxygen-centred radicals, and it is also probably the radical that is most frequently misassigned. Formation from water requires very high energy, and is normally accomplished by ionizing radiation. It is probably the most reactive of the radical species formed in the radiolysis of aqueous solutions, the other major species being solvated electrons and hydrogen atoms (12)–(14).

$$H_2O \rightarrow H_2O^+ + e^-, \tag{12}$$

$$H_2O + H_2O^+ \rightarrow OH^{\cdot} + H_3O^+, \tag{13}$$

$$e^- + H_3O^+ \rightarrow H^{\cdot} + H_2O. \tag{14}$$

It can be formed chemically from OH^- ions, especially if these are only weakly solvated, when they become good electron donors, as stressed above. Generation from H_2O_2 is the most common chemical method, with the use of ultraviolet light, which ruptures the O—O bond, or certain transition metal complexes which are good electron donors, such as Fe^{II} or Ti^{III} (15).

$$H_2O_2 + Fe^{2+}_{aq} \rightleftharpoons FeOH^{2+}_{aq} + OH^{\bullet}. \qquad (15)$$

Many ferrous complexes are equally able to generate $OH^{\bullet}$ radicals from H_2O_2, so this combination can be biologically dangerous.

The pK_a of $OH^{\bullet}$ radicals is close to that of water, so $O^{-\bullet}$ anions are unlikely to be of importance in biochemistry. (The latter are formed readily in the radiolysis of aqueous alkaline glasses, and have an e.s.r. spectrum again determined by the nature of the solvation of the ion (Blandamer *et al.* 1964; Symons 1982*a*, *b*). One important chemical property is their ability to react with dioxygen to form the ozonide radical ion, $O_3^{-\bullet}$ (Atkins *et al.* 1962), which, because of its relatively high stability, may be of some importance.)

The SOMO for $OH^{\bullet}$ is similar to that for O_2^-, and again, e.s.r. spectra are controlled by hydrogen bonding for aqueous systems. When formed by radiolysis of ice crystals, well defined e.s.r. spectra are obtained (Symons 1982*a*, *b*; Brivati *et al.* 1967), with the expected large positive shift in g_z, and a large hyperfine coupling to the proton ($A_{iso} \approx (-)\ 27$ G). However, in frozen glassy systems this 'parallel' (g_z) feature is broadened, often so strongly that it is completely lost (Riederer *et al.* 1983). This result is important because it underlines the control exerted by solvation. As with O_2^-, and for the same reasons, no spectrum for $OH^{\bullet}$ radicals in the liquid phase has ever been detected.

It is therefore helpful to use a spin trap to establish the presence of $OH^{\bullet}$ radicals in solution, especially as the rapid-freeze technique has not proved satisfactory. One major problem is the high reactivity of $OH^{\bullet}$ radicals. They are expected to react indiscriminately at close to diffusion-controlled rates, so high concentrations of traps are required to compete with other substrates. Furthermore, these secondary radicals are expected to react with the traps to give other nitroxide radicals whose spectra may obscure that for the $OH^{\bullet}$ adducts. Fortunately, if $OH^{\bullet}$ radicals react with large biopolymers, the resulting bio-radicals may not react efficiently with the traps, and the adducts, once formed, may give very broad-lined spectra because of slow tumbling rates. If several nitroxide radicals are formed concurrently, and are sufficiently stable, they can be separated by using high-pressure liquid chromatography (Moriya *et al.* 1982; Makino & Riesz 1982), greatly facilitating spectral analysis.

There are many examples of the use of spin traps to establish the presence of $OH^{\bullet}$ radicals, and some attempts at quantification. A few selected examples are given in the references (Finkelstein *et al.* 1980; Marriott *et al.* 1980; Green *et al.* 1979; Floyd 1982; Berlin & Haseltine 1981; Komiyama *et al.* 1982). It seems certain that the best trap so far investigated is DMPO (scheme 1), whose hydroxy adduct has a distinctive four-line spectrum (figure 8) and is relatively long-lived.

There are, however, at least two potential dangers in concluding that detection of this adduct provides clear proof that $OH^{\bullet}$ radicals were the precursors. One is that nitrones have relatively low ionization potentials, and might become oxidized to the radical cations. These would be expected to react rapidly with water to give the —OH adduct. Another is that certain hydroxylating agents are thought to be capable of hydroxylating the traps without actual release of free $OH^{\bullet}$ radicals (Lown & Chen 1981) and further chemical tests are needed to distinguish between such possibilities. Thus pitfalls abound: nevertheless, there can be little doubt that in several cases free $OH^{\bullet}$ radicals are genuinely involved and can be identified by spin trapping. However, I am personally dubious about some examples in which relatively large, delocalized radicals are invoked as being precursors to the formation of free $OH^{\bullet}$ radicals (Floyd 1982; Berlin & Haseltine 1981; Komiyama *et al.* 1982).

The $OH^{\bullet}$ radical is often described as being 'electrophilic'. Indeed, it is common practice to classify radicals as 'nucleophilic' or 'electrophilic'. This does not relate to the contrast outlined above, in which radicals such as O_2^- can react as a radical or as a nucleophile. Rather, it relates to the tendency for the radical to donate its unpaired electron to the substrate, or to accept an electron from the substrate, as the transition state is approached. The situation is shown schematically in figure 13, for two extreme types of radicals. One, which I prefer to classify as an electron-donor radical, tends to transfer its electron into the LUMO (lowest unoccupied, or virtual molecular orbital) while the other, an electron-acceptor radical, tends to accept an electron-pair from the HOMO (highest occupied molecular orbital) of the substrate while donating its own electron simultaneously, as shown in figure 13*b*. Radicals such as methyl commonly fall between these extremes, while $OH^{\bullet}$ radicals, or radicals such as ${}^{\bullet}CF_3$, can be classified as electron-acceptor species. In contrast, the radical ${}^{\bullet}BH_3^-$ is a good electron-donor radical.

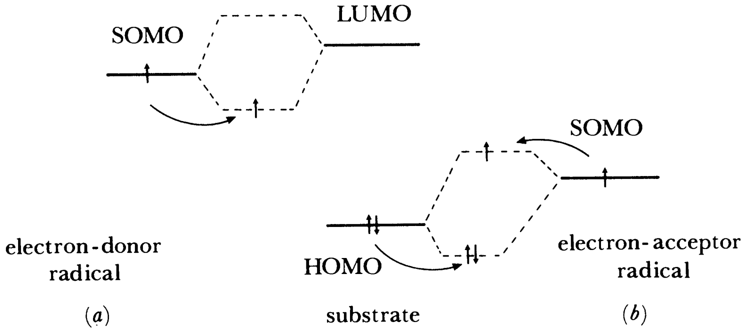


FIGURE 13. Schematic representation of the interaction between the LUMO of a substrate with an electron-donor radical (*a*), and the HOMO of a substrate with an electron-acceptor radical (*b*).

The hydroxyl radical is clearly an 'electron-acceptor' radical. In one limit, thought by some to make a major contribution towards its reactivity, a reaction such as hydrogen-atom abstraction can be thought of as proceeding via an initial electron transfer, followed by a very rapid proton transfer, as in (16).

$$\mathrm{OH^{\bullet}} + \mathrm{A{-}H} \overset{(a)}{\rightleftharpoons} \mathrm{OH^-} + {}^{\bullet}\mathrm{AH^+} \xrightarrow{(b)} \mathrm{H_2O} + \mathrm{A^{\bullet}}. \qquad (16)$$

Both (16*a*) and (16*b*) are often favourable processes, but to what extent there is any real division between stages (*a*) and (*b*) is unknown.

5. ONE-ELECTRON AND TWO-ELECTRON PROCESSES

It has been realized for very many years that reactions such as the conversion of dioxygen into hydrogen peroxide may proceed by one- or two-electron routes. Biomolecules are well equipped to react by either route. For example, haem-iron can be present in the two-, three-, or four-valent states, and molybdenum in xanthine oxidase moves from Mo^{VI} to Mo^{IV} without the *necessary* involvement of Mo^{V}. However, there are clearly occasions when reactive radicals, especially $OH^{\cdot}$, are useful for rapid, indiscriminate, attack on unwanted species. Thus both routes need to be available, and controls are needed which can call on one while suppressing the other, to suit demand.

6. Reactions with transition metal complexes

When $OH^{\cdot}$ radicals react directly with the metal centre, rather than with one of the ligands, they normally lose all radical character, the SOMO becoming part of the metal d-orbital system, as in (17).

$$Fe^{2+} + OH^{\cdot} \rightarrow Fe^{3+}—OH^{-}. \qquad (17)$$

In contrast, reactions with O_2^- may result in a complex in which the SOMO remains largely on the ligand, which therefore retains considerable radical character. This depends intimately on orbital availability and on relative electronegativities. For example, e.s.r. studies have shown that for the $Fe^{III}–O_2^-$ haemoglobin derivative, the SOMO is extensively delocalized onto iron, whereas for the isoelectronic cobalt derivative it is largely confined to dioxygen (Symons & Petersen 1968; Dickinson & Chien 1973; Dickinson & Symons 1983).

7. Other inorganic radicals

(*a*) *Solvated electrons*

In general, excess electrons are accommodated in chemical systems within antibonding orbitals of electron-affinic molecules. However, if these are not immediately available, they may become 'solvated'. This means that they become quite strongly localized in a stationary orbital which is probably unique in having no *central* nucleus. These species are closely related to F-centres in alkali halide crystals, whose structures have been unambiguously defined by e.s.r. spectroscopy (Delbecq 1963). A first-order description of an F-centre can be simply viewed as one in which a single halide ion is removed from the crystal lattice, being replaced by an electron that remains quite strongly localized in an s-type orbital within the anion site (Claxton *et al.* 1966). Similarly, my view of an aquated electron is obtained by imagining the removal of, say, a chloride ion from water, and replacing it by an electron, leaving the local structure approximately unchanged (Symons 1976, 1981). This simple model has received extensive experimental support, but is not universally accepted (Golden & Tuttle 1978; Stradowskii & Hamill 1976).

It is unlikely that solvated electrons are ever involved in biological reactions except when exposed to ionizing radiation. They are not well characterized by e.s.r. spectroscopy, because rapid exchange processes completely remove all hyperfine coupling to solvent nuclei. They are, however, characterized by an intense visible absorption band, by which their reactions can be monitored in pulse-radiolysis studies.

(*b*) *Hydrogen atoms*

These are also not normally invoked as intermediates in biological reactions. As stressed above, they are an important intermediate in the radiolysis of water, and are well characterized in e.s.r. spectroscopy by a narrow 508 G doublet. There is an interesting chemical reaction that relates hydrogen atoms and electrons, namely (18) (Baxendale & Hughes 1958).

$$H^{\cdot} + OH^{-}_{solv} \rightleftharpoons H_2O + e^{-}_{solv}. \qquad (18)$$

Although we have direct e.s.r. evidence in favour of this equilibrium (Symons & Zimmerman 1976), I still find it surprising!

(*c*) *Chlorine-centred radicals*

Hydroxyl radicals are able to form weak adducts with chloride ions (19) (Catton & Symons 1969*a*; Ginns & Symons 1972).

$$OH^{\cdot} + Cl^{-} \rightleftharpoons HO\overset{\cdot}{-}Cl^{-}. \qquad (19)$$

These are classified as σ^* radicals because the SOMO is the antibonding O—Cl σ-orbital. I have no idea to what extent their formation may modify reactions of $OH^{\cdot}$ radicals in biological systems. They readily react with a second chloride ion to give the more stable Cl_2^- radical, which also has a σ^* SOMO (20).

$$Cl^{-} + HOCl^{-} \rightleftharpoons Cl\overset{\cdot}{-}Cl^{-} + OH \qquad (20)$$

Both are potential chlorine-atom donors and may chlorinate rather than hydroxylate. $HOCl^-$ is an electron donor, giving the biologically important HOCl molecule and ClO^- anion, as well as being an electron acceptor.

Electron donation by ClO^- may also be a reaction of some biological significance. Unfortunately, the $ClO^{\cdot}$ radical, like the isostructural O_2^- radical has a degenerate π^* SOMO and hence has never been detected in the liquid phase. I know of no spin-trapping work on $ClO^{\cdot}$ radicals. Addition could occur via Cl or O addition: the former should give a resolved quartet hyperfine coupling from chlorine, but the latter is more probable.

A radical formed by photolysis of chlorine dioxide in acidic glasses, originally thought to be $ClO^{\cdot}$ (Atkins *et al.* 1962) was later shown to be the rearranged dioxide, $ClOO^{\cdot}$ (Eachus *et al.* 1968). The e.s.r. spectrum for this radical exhibits a well defined hyperfine coupling to chlorine nuclei and only a small g value variation, showing that it is strongly bent, as expected. A species showing very large g shift and a greater coupling to chlorine has recently been identified as genuine $ClO^{\cdot}$ (Trainer *et al.* 1983).

(*d*) $NO^{\cdot}$ *and* $^{\cdot}NO_2$ *radicals*

Both $NO^{\cdot}$ and $^{\cdot}NO_2$ are relatively stable inorganic radicals which might be expected to form in biological systems under certain circumstances. The $NO^{\cdot}$ radical is of particular importance in its ability to act as a ligand, especially to haem iron. Thus, for example, deoxymyoglobin and haemoglobin readily react with NO to give the nitrosyl derivatives. As with dioxygen complexes, there is appreciable bonding, and the SOMO moves extensively into metal 3d-orbitals. However, the resulting e.s.r. spectra are characterized by triplet splitting from the ^{14}N nucleus [$I = 1$], showing that delocalization is extensive (Rein *et al.* 1972). It is interesting to note that e.s.r. signals from nitrosyl haemproteins were detected in early studies of tumours, when it was hoped that e.s.r. spectroscopy might be an important tool in studies of cancers. Initially, it was hoped that these signals established the importance of radicals (Vithayathil *et al.* 1965), but later the signals were recognized as being characteristic of the Fe—NO unit (Chiang *et al.* 1972), formed from fortuitously added nitrate or nitrite ions.

(*e*) *The* $^{\cdot}\mathrm{SO}_2^-$ *and* $^{\cdot}\mathrm{SO}_3^-$ *radicals*

The former radical is probably the reactive species in reactions of dithionites, because it is formed reversibly from $\mathrm{S_2O_4^{2-}}$ in aqueous solutions at room temperature, giving rise to a well defined e.s.r. spectrum (Atkins *et al.* 1964). The latter radical has been implicated in reactions involving sulphite ions (or $\mathrm{HSO_3^-}$) and prostaglandin synthase (Mottley *et al.* 1982). In this work, it was detected indirectly by using a DMPO trap. In fact, $^{\cdot}\mathrm{SO}_3^-$ radicals have a remarkably narrow solid-state e.s.r. feature, and give rise to a narrow liquid-phase singlet that is readily detectable (Ozawa & Kwan 1983). Hence, this is a radical that might well prove to be directly detectable by e.s.r. spectroscopy if it were formed in significant yields.

8. COMPARISON OF OXYGEN- AND SULPHUR-CENTRED RADICALS

Although purely inorganic sulphur analogues, such as $\mathrm{S}_2^{-\cdot}$, $\mathrm{HS}_2^{\cdot}$ and $\mathrm{HS}^{\cdot}$ are not, so far as I am aware, thought to be of importance in most biological systems, various alkyl derivatives are almost certainly involved. It may therefore be of interest to draw a few comparisons between the two groups.

First, there is a major difference in reactivity between $\mathrm{OH}^{\cdot}$ and $^{\cdot}\mathrm{SH}$ or $^{\cdot}\mathrm{OR}$ and $^{\cdot}\mathrm{SR}$ radicals. Returning to reaction (16), the $^{\cdot}\mathrm{SH}$ radical has a lower electron affinity and SH^- has a lower proton affinity than OH^-. Hence $^{\cdot}\mathrm{SH}$ or $^{\cdot}\mathrm{SR}$ radicals are generally less reactive than $\mathrm{OH}^{\cdot}$ radicals. Conversely, RSH molecules are good hydrogen-atom donors, and it seems probable that they play an important 'healing' role in biological systems where biomolecules, especially DNA, have suffered attack by radicals. Not only are $\mathrm{RS}^{\cdot}$ radicals relatively stable, they also have a mechanism of protecting themselves that is not apparently available to oxygen-centred radicals.

(*a*) σ^* *radicals*

A major contrast between the two groups is the relative prevalence of σ^* sulphur species such as $\mathrm{RS}\overset{\cdot}{-}\mathrm{SR}^-$, $\mathrm{R_2S}\overset{\cdot}{-}\mathrm{SR}_2^+$ and probably $\mathrm{RS}\overset{\cdot}{-}\mathrm{SR}_2$ radicals. All these, like the radicals $\mathrm{HO}\overset{\cdot}{-}\mathrm{Cl}^-$ and $\mathrm{Cl}\overset{\cdot}{-}\mathrm{Cl}^-$ mentioned above, have σ^* SOMOs. This situation is illustrated in figure 14. (In this figure, the normal strong σ-bonding is contrasted with the weakening that results on electron gain into the σ^*-orbital, and with loss of one electron from the σ-orbital. Although radicals of the latter type are known (Wang & Williams 1981; Symons 1982), I know of no examples in which they are involved in biological systems.)

It is curious that the analogous oxygen-centred σ^* radicals do not appear to have any chemical significance. Some current theories support the idea that oxygen-centred σ^* radicals are likely to be far less stable than corresponding sulphur-centred σ^* radicals, but others suggest the reverse. This means that there are not likely to be any obvious explanations.

These σ^* radicals can be formed by oxidative or reductive mechanisms, as in the reactions (21) + (22) and (23).

$$\mathrm{RS}^- \rightarrow \mathrm{RS}^{\cdot} + \mathrm{e}^-, \tag{21}$$

$$\mathrm{RS}^{\cdot} + \mathrm{RS}^- \rightleftharpoons \mathrm{RS}\overset{\cdot}{-}\mathrm{SR}^-, \tag{22}$$

$$\mathrm{RS{-}SR} + \mathrm{e}^- \rightarrow \mathrm{RS}\overset{\cdot}{-}\mathrm{SR}^-, \tag{23}$$

$$\mathrm{RS}^{\cdot} + \mathrm{R_2S} \rightleftharpoons \mathrm{RS}\overset{\cdot}{-}\mathrm{SR}_2. \tag{24}$$

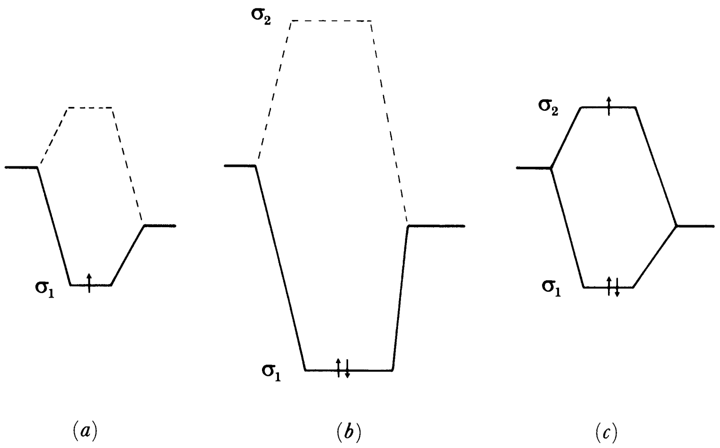


FIGURE 14. Qualitative energy level diagram for σ_1^1 cations (*a*) and $\sigma_1^2\sigma_2^1$ anion-radicals (*c*) relative to that for the parent molecule (*b*).

Comparing reactions of OH˙ or RO˙ radicals with those of RS˙ radicals, the former readily extract hydrogen from organic compounds, whereas the latter tend to react as in (22) or (24) to form σ^* radicals, which are greatly stabilized, and are unlikely to cause damage to biomolecules.

There is considerable controversy regarding the significance of radicals formulated as $RS\dot{-}SR_2$ above (Symons 1974; Nelson *et al.* 1977, 1978). The e.s.r. spectra of intermediates widely encountered in sulphur-radical studies, characterized by features at $g \approx 2.060$, $g \approx 2.025$ and $g \approx 2.00$, were originally thought to be a result of RS˙ radicals (Symons 1974). However, it is now clear that this contains two inequivalent sulphur nuclei (Hadley & Gordy 1974). The idea that this species is the expected $RS\dot{-}SR_2$ σ^* radical is attractive mechanistically (Symons 1974; Nelson *et al.* 1977, 1978), but there are strong reasons for believing that RSS˙ radicals may be responsible (Hadley & Gordy 1974). The current situation has recently been discussed (Symons 1985). It may be that by a quirk of nature, both types of radical have very similar spectra.

Nature profits from these differences by using RSH compounds to heal the wounds caused by radicals such as OH˙. The RSH molecules act as hydrogen donors, and provided the RS˙ radicals are stabilized as in (22) or (24) before they react with oxygen, trouble is averted. Reaction with oxygen, to give RS—OO˙ radicals, is thought to be of significance although present evidence is not compelling (Copeland 1975). If it does occur, it converts a relatively stable radical into a more reactive species which can no longer acquire added stability by forming a σ^* species.

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Discussion

H. SIES (*Institut für Physiologische Chemie I, Universität Dusseldorf, F.R.G.*). I was interested by Dr Symons's remark about the different chemical reactivity of singlet molecular oxygen in aprotic solvents, and that singlet oxygen might be more soluble in non-aqueous phases than triplet oxygen. Would he care to speculate on the biological significance?

M. C. R. SYMONS. My comment is purely speculative and centres on the idea that solvated singlet O_2 might have an asymmetric distribution of charge with, say, $\delta+$ in π_x and $\delta-$ in π_y. Thus, singlet O_2 may be better solvated by water than normal triplet O_2. Thus, in fact, I am suggesting that the singlet form should be more soluble in the aqueous phase than triplet oxygen.

I am afraid I know of no clear examples of this predicted situation as yet.