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and

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Conventions

The abbreviations, conventions and symbols used in these Essays are those specified by the Editorial Board of *The Biochemical Journal* in *Policy of the Journal and Instructions to Authors* (revised 1976 *Biochem J.* **153**, 1–21 and amended 1978 *Biochem J.* **169**, 1–27). The following abbreviations of compounds, etc., are allowed without definition in the text.

ADP, CDP, GDP, IDP, UDP, XDP, dTDP: 5′-pyrophosphates of adenosine, cytidine, guanosine, inosine, uridine, xanthosine and thymidine
AMP, etc.: adenosine 5′-phosphate, etc.
ATP, etc.: adenosine 5′-triphosphate, etc.
CM-cellulose: carboxymethylcellulose
CoA and acyl-CoA: coenzyme A and its acyl derivatives
Cyclic AMP etc.: adenosine 3′ : 5′-cyclic phosphate etc.
DEAE-cellulose: diethylaminoethylcellulose
DNA: deoxyribonucleic acid
Dnp-: 2,4-dinitrophenyl-
Dns-: 5-dimethylaminonaphthalene-1-sulphonyl-
EDTA: ethylenediaminetetra-acetate
FAD: flavin-adenine dinucleotide
FMN: flavin mononucleotide
GSH, GSSG: glutathione, reduced and oxidized
NAD: nicotinamide-adenine dinucleotide
NADP: nicotinamide-adenine dinucleotide phosphate
NMN: nicotinamide mononucleotide
P_i, PP_i: orthophosphate, pyrophosphate
RNA: ribonucleic acid (see overleaf)
TEAE-cellulose: triethylaminoethylcellulose
tris: 2-amino-2-hydroxymethylpropane-1,3-diol

The combination NAD^+, NADH is preferred.

The following abbreviations for amino acids and sugars, for use only in presenting sequences and in Tables and Figures, are also allowed without definition.

Amino acids

Ala: alanine	Asx: aspartic acid or asparagine (undefined)	Cys or Cys: Cystine (half)
Arg: arginine		
Asn*: asparagine	Cys: Cysteine	Gln†: glutamine
Asp: aspartic acid		Glu: glutamic acid

* Alternative, Asp(NH_2) † Alternative, Glu(NH_2)

Biography

Raymond Bonnett graduated from Imperial College and studied for his Ph.D. at Cambridge, working with Sir Alexander Todd and A. W. Johnson on the chemistry of vitamin B_{12}. After a year with R. B. Woodward's team working on the synthesis of chlorophyll, and two years at the University of British Columbia, he returned to the United Kingdom. His major interests are the chemistry and biochemistry of tetrapyrroles and related compounds. Since 1976 he has held the Chair of Organic Chemistry at Queen Mary College (University of London).

B. Foltmann graduated as a pharmaceutical chemist from the Royal Danish School of Pharmacy, Copenhagen. He then worked in the Chr. Hansens Laboratory Ltd., Copenhagen, the Carlsberg Laboratory and the MRC Laboratory of Molecular Biology, Cambridge, and obtained his Dr. scient. from the University of Copenhagen in 1966. He then moved to the Faculty of Medicine, University of Copenhagen, and has been Professor of Biochemical Genetics in the Faculty of Science since 1970. His main contribution to biochemistry has been the mapping of the properties of prochymosin and chymosin and determination of their primary structures. His present research is on the comparison of the properties of other gastric proteases and their zymogens.

Alan Paine studied Biochemistry at Queen Elizabeth College London, received his Ph.D. at University College Hospital Medical School London, and is currently a member of the scientific staff of the M.R.C. Toxicology Unit, Carshalton, Surrey. His research interests are centred around the mechanism(s) of induction of hepatic cytochrome P-450. He is also interested in understanding mechanisms of hepatotoxicity at a cellular level.

Glx: glutamic acid or glutamine (undefined)
Gly: glycine
His: histidine
Hyl: hydroxylysine
Hyp: hydroxyproline
Ile: isoleucine
Leu: leucine
Lys: lysine
Met: methionine
Orn: ornithine
Phe: phenylalanine
Pro: proline
Ser: serine
Thr: threonine
Trp: tryptophan
Tyr: tyrosine
Val: valine

Sugars

Ara: arabinose
dRib: 2-deoxyribose
Fru: fructose
Fuc: fucose
Gal: galactose
Glc*: glucose
Man: mannose
Rib: ribose
Xyl: xylose

* Where unambiguous, G may be used.

Abbreviations for nucleic acid used in these essays are:

mRNA: messenger RNA
nRNA: nuclear RNA
rRNA: ribosomal RNA
tRNA: transfer RNA

Other abbreviations are given on the first page of the text.

References are given in the form used in *The Biochemical Journal*, the last as well as the first page of each article being cited and, in addition, the title. Titles of journals are abbreviated in accordance with the system employed in the *Chemical Abstracts Service Source Index* (1975) and its Quarterly Supplement (American Chemical Society).

Enzyme Nomenclature

At the first mention of each enzyme in each Essay there is given, whenever possible, the number assigned to it in *Enzyme Nomenclature: Recommendations (1978) of the Nomenclature Committee of the International Union of Biochemistry on the Nomenclature and Classification of Enzymes*, published for the International Union of Biochemistry by Academic Press, New York and London, 1979. Enzyme numbers are given in the form EC 1.2.3.4. The names used by authors of the Essays are not necessarily those recommended by the International Union of Biochemistry.

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Oxygen Activation and Tetrapyrroles†

RAYMOND BONNETT

Department of Chemistry, Queen Mary College, Mile End Road, London E1 4NS, England

Superoxide dismutase Superoxide :superoxide oxidoreductase, EC 1.15.1.1.
Xanthine oxidase Xanthine :oxygen oxidoreductase, EC 1.2.3.2.
Catalase Hydrogen-peroxide :hydrogen-peroxide oxidoreductase, EC 1.11.1.6.
Peroxidase Donor :hydrogen-peroxide oxidoreductase, EC 1.11.1.7.
Cytochrome *c* oxidase Ferrocytochrome *c* :oxygen oxidoreductase, EC 1.9.3.1.
D-Amino-acid oxidase D-amino-acid :oxygen oxidoreductase (deaminating). EC 1.4.3.3.
Dopamine *β*-monooxygenase 3,4-Dihydroxyphenylethylamine, ascorbate:oxygen oxidoreductase (5-hydroxylating), EC 1.14.17.1.
Tryptophan 2,3-dioxygenase L-Tryptophan :oxygen 2,3-oxidoreductase (decyclizing), EC 1.13.11.11.
Cytochromes P-450 in variety Flavoprotein-linked monooxygenase—RH, reduced-flavoprotein :oxygen oxidoreductase (RH-hydroxylating), EC 1.14.14.1. Camphor 5-monooxygenase—Camphor, reduced-putidaferredoxin:oxygen oxidoreductase (5-hydroxylating), EC 1.14.15.1. Arene monooxygenase (epoxidizing)—Naphthalene, hydrogen-donor :oxygen oxidoreductase (1,2-epoxidizing), 1.14.99.8.
Haem oxygenase (decyclizing) Haem, hydrogen-donor :oxygen oxidoreductase (*α*-methene-oxidizing, hydroxylating), EC 1.14.99.3.
Biliverdin reductase Bilirubin :$NAD(P)^+$ oxidoreductase, EC 1.3.1.24.
Glutathione peroxidase Glutathione :hydrogen-peroxide oxidoreductase, EC 1.11.1.9.
Chloride peroxidase Chloride :hydrogen-peroxide oxidoreductase, EC 1.11.1.10.

† Based on an introductory lecture given at a meeting on this topic held by the Tetrapyrrole Discussion Group in Leeds on 29th March 1979.

"Having procured a lens of twelve inches diameter, and twenty inches focal distance, I proceeded with great alacrity to examine, with the help of it, what kind of air a great variety of substances, natural and factitious, would yield . . . On the 1st August, 1774, I endeavoured to extract air from *mercurius calcinatus per se*; and I presently found that, by means of this lens, air was expelled from it very readily. Having got about three or four times as much as the bulk of my materials, I admitted water to it, and found that it was not imbibed by it. But what surprised me more than I can well express, was, that a candle burned in this air with a remarkably vigorous flame. . . . Who can tell but that, in time, this pure air may become a fashionable article of luxury. Hitherto only two mice and myself have had the privilege of breathing it."

Joseph Priestley, on discovering oxygen (dephlogisticated air) in 1774

"13 Novr. 1847

9235. *Oxygen* in coal gas was very magnetic and the experiment was very beautiful. The ammonia, in uniting with the muriatic acid of the issuing oxygen, made a cloud which fell well and quickly; but on making the magnet active, the cloud instantly ran back, and accumulated about the poles, occupying the magnetic field, so that the pole ends were hid in a cloud of smoke an inch or more in diameter. When the magnet was thrown out of action, this instantly fell and was cleared away, or if allowed to fall only a little and then the magnetic force renewed, it rushed back again to the magnetic field."

Michael Faraday, Diary

I. Introduction

Oxygen is a substance of common experience, a substance the use of which we share as we live together on the surface of our planet. It is a fascinating substance. It is the most abundant element in the earth's mantle: taking ocean and crust together, then 50% by weight is oxygen. For the total earth, the figure is about 28% oxygen, mostly in the form of oxides.

In its natural occurrence, oxygen is almost, but not quite, monoisotopic (Table 1).[1] The natural isotopes can be separated (^{18}O by fractional distillation

TABLE 1

Natural isotopic abundance of oxygen in air

Isotope	Abundance (%)
^{16}O	99·759
^{17}O	0·037
^{18}O	0·204

of water, ^{17}O by thermal diffusion of oxygen gas), but these procedures are expensive, and so are the products. Oxygen-18 is useful in labelling experiments: it is not radioactive, of course, and so the fate of the label must be determined using mass spectrometry. Oxygen-17 is of particular interest because, unlike the other natural isotopes, it has nuclear spin ($I = 5/2$), and so ^{17}O-nuclear magnetic resonance spectroscopy has developed. Unfortunately, ^{17}O is the least abundant isotope, and it is likely to remain rather expensive. At the time of writing, water with 50% enrichment of this isotope is £200 per gram. There is no really convenient radioisotope: oxygen-15, the longest lived, has a half-life of only 124 sec. It has occasionally been used, nonetheless. For example, it was recently employed in an attempt to discover the fate of the burst of oxygen consumed during phagocytosis.[2]

The principal allotropes of oxygen are dioxygen, O_2, and ozone, O_3 (Fig. 1). A third allotrope, O_4, has been said to be formed in liquid oxygen under high pressure, but need not detain us here. Ozone is thermodynamically less stable than dioxygen (Fig. 1). It can be regarded as an activated form of oxygen, but, as far as I am aware—and seaside air notwithstanding—it plays no direct part in the chemistry of normal metabolic processes. Ozone has, however, a most important passive role, for as a layer in the upper atmosphere it acts as a sun-screen ($\lambda_{max} = 253{\cdot}7$ nm),[1] cutting out from solar radiation the high-energy ultraviolet region which would otherwise be absorbed by, and cause photochemical changes in, cell constituents such as DNA. Life would be very different and, one suspects, much less pleasant, without the ozone layer.

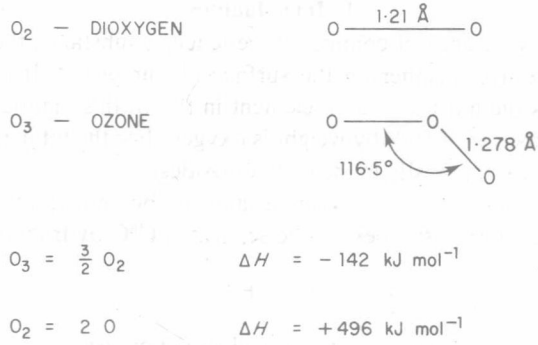

Fig. 1. Allotropic forms of oxygen. (Thermodynamic and geometrical parameters from Cotton, F. A. & Wilkinson, G. (1980). *Advanced Inorganic Chemistry*. Fourth edition, Wiley, New York & Chichester.)

Most of this essay is about dioxygen and species derived from it: I shall use the term dioxygen, which is the correct chemical name for molecular oxygen, whenever to do so removes an ambiguity. In what follows we shall first of all look, rather briefly, at activated oxygen species of possible biological significance. We then proceed to consider a rich variety of biological interactions between oxygen species and tetrapyrroles.

II. Oxygen

A. REDOX AND ACID–BASE RELATIONSHIPS AMONG OXYGEN SPECIES

(*1*) *Monooxygen species*

It is useful, because it throws light on chemical relationships and at the same time sorts out problems of nomenclature, to set out the various possible oxygen species on a grid in which reduction occurs in a stepwise fashion going from left to right, and protonation likewise from top to bottom. This is done for monooxygen systems in Fig. 2.

The species here are generally familiar enough, and from time to time various of them have been advanced as activated forms of oxygen involved in biological systems. Atomic oxygen is also called "oxene", a name coined to make reference to the nitrene and carbene systems with which it is analogous. Formally, HO^+ is protonated (singlet) atomic oxygen. These are both high-energy species, extremely reactive, and unlikely to exist in a free state in living tissues. However, it is quite conceivable that a biochemical system may be able to generate at a given site a monooxygen adduct with the reactivity of

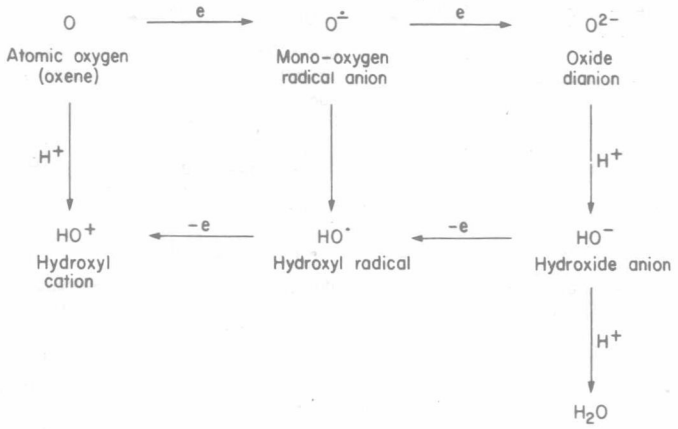

Fig. 2. Formal relationships between monooxygen species.

one of these, without it ever becoming free from the enveloping reaction centre. Such an adduct, showing reactions like that of oxene, is called *oxenoid.*

The situation with hydroxyl radicals is not clear cut. Such radicals are well known chemically, but whether they are formed in a controlled way in a biological system is a matter of some controversy. Babior[3] has recently advanced the view that the killing of bacteria by specialized leucocytes, a process which is accompanied by an increased oxygen consumption, is due to hydroxyl radicals generated by the Haber–Weiss reaction catalysed by iron(II):

$$O_2^{\cdot-} + H_2O_2 \xrightarrow{Fe(II)} HO^{\cdot} + HO^- + O_2$$

Clearly a package containing this mixture will be lethal for the bacterium: the conceptual difficulty is that we have to provide a wrapper for the package.

(2) *Dioxygen species*

An analogous grid for dioxygen species is set out in Fig. 3. Some of the species here are perhaps less familiar. The hydroperoxonium, HO_2^+, is formally protonated dioxygen: it has been recognized in the mass spectrometer in the fragmentation of hydrogen peroxide after electron impact[4] (OH^+ and other cations are also seen under these conditions). The dioxygenyl radical cation, O_2^+, is known, and salts of it can be isolated. Thus dioxygenyl hexafluoroplatinate can be formed by oxidizing oxygen (!) with platinum hexafluoride.[5]

$$O_2 + PtCl_6 \longrightarrow O_2^+[PtCl_6]^-$$

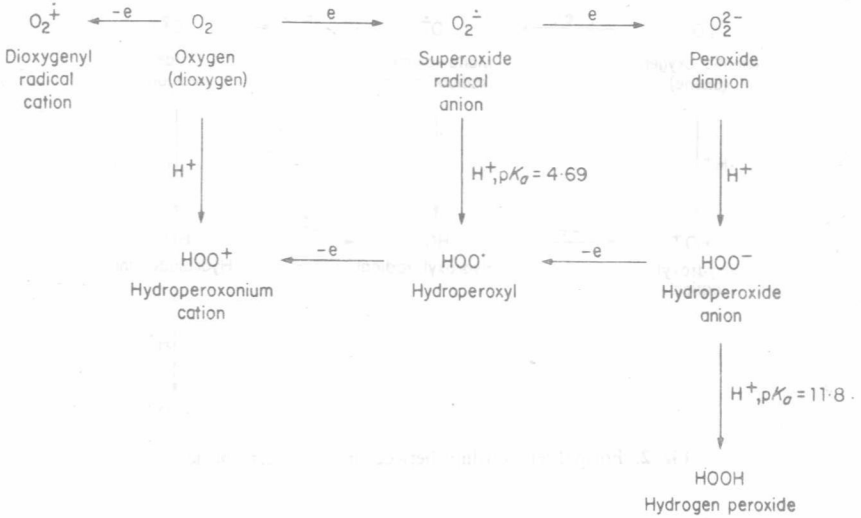

Fig. 3. Formal relationships between dioxygen species.

Although hydroperoxonium and dioxygenyl cations are most unlikely to be accessible under biological conditions, certain other of the species in Fig. 3—for example, superoxide and peroxide—are well known in this context. Indeed many tissues appear to be provided with enzymes the purpose of which seems to be to guard against the adventitious formation of these species (Section II.D.2; Section III.G). The hydroperoxyl radical is simply protonated superoxide: at pH 7 the superoxide anion radical predominates (>99%).

B. ELECTRONIC STRUCTURE

The paramagnetism of dioxygen was discovered by Faraday in 1847. Since paramagnetism normally implies that not all electrons are paired, the structure of dioxygen cannot be represented correctly as O=O. Hence, as every first-year chemist (and, hopefully, biochemist) knows, an alternative to the simple valence bond theory of bonding has to be sought. An approach which gives a more satisfactory picture employs the molecular orbital theory in one of its forms (linear combination of atomic orbitals molecular orbital, LCAOMO, approach). Development of this theory shows that the atomic orbitals of the valence shells of the two oxygen atoms involved in bonding interact to give a series of molecular orbital energy levels, as shown in Fig. 4. It is then necessary to feed in the twelve valence electrons (i.e. $2 \times 2\,s^2\,p^4$) starting with the molecular orbital of lowest energy (arrows in Fig. 4 represent electrons). All proceeds smoothly until the last two electrons are introduced into the antibonding π orbitals (π_x^* and π_y^*). Here there are two orbitals possessing the

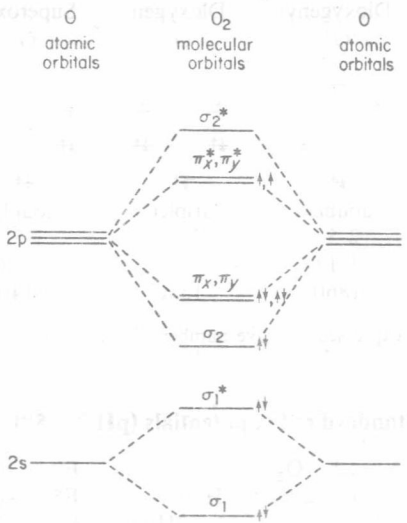

Fig. 4. Molecular orbital energy level diagram for dioxygen.

same energy and, because of electron repulsion, each electron occupies a different orbital, and the spins are parallel. Hence dioxygen has two unpaired spins.

In chemical theory each electron is said to have a spin $\frac{1}{2}$. Total spin, S, is the sum of such spins, and the multiplicity is given by $2S + 1$. Thus dioxygen in its ground state (i.e. ordinary molecular oxygen) has a multiplicity of three (i.e. $2(\frac{1}{2} + \frac{1}{2}) + 1$), and is referred to as a triplet. Most organic molecules that we meet are diamagnetic and have all their electrons paired. Their total spin is zero and multiplicity is one: they are singlets in their ground state.

The electronic configuration and some properties of redox species of dioxygen are given in Fig. 5. As we proceed from dioxygenyl to peroxide by stepwise reduction, electrons are being added to the antibonding π orbitals (π_x^* and π_y^*). The bond order (number of pairs of electrons in bonding orbitals minus number of pairs of electrons in antibonding orbitals) decreases, the bond length increases, and the stretching frequency decreases, at each successive stage of reduction.

The molecular orbital approach also offers a description of the electronically excited states of dioxygen. Although the direct excitation of ground state dioxygen requires rather special circumstances, indirect excitation by energy transfer via a sensitizer such as methylene blue, rose bengal, or a porphyrin (Section IV) is readily accomplished. The two lowest excited states are both singlets (Fig. 6) but the higher-energy species (designated $^1\Sigma_g^+$) is so short-lived in solution that for practical purposes reaction in biological systems is expected

	Dioxygenyl O_2^+	Dioxygen O_2	Superoxide O_2^-	Peroxide O_2^{2-}
1. Electronic configuration σ_2^*	—	—	—	—
π^*	↑ —	↑ ↑	↑↓ ↑	↑↓ ↑↓
π	↑↓ ↑↓	↑↓ ↑↓	↑↓ ↑↓	↑↓ ↑↓
σ_2	↑↓	↑↓	↑↓	↑↓
2. Multiplicity	doublet	triplet	doublet	singlet
3. Bond order	2·5	2·0	1·5	1·0
4. Bond length (Å)	1·12	1·21	1·33	1·49
5. $\bar{\nu}_{O-O}$ cm^{-1}*	1860	1550	1140	770

* Stretching frequency, expressed as wave numbers, for the vibration of the oxygen–oxygen bond.

Standard redox potentials (pH 7, 25°C)

$$O_2 + e \rightleftharpoons O_2^- \qquad E° = -0{\cdot}4 \text{ volts}$$
$$O_2^- + 2H^+ + e \rightleftharpoons H_2O_2 \qquad E° = +0{\cdot}90 \text{ volts}$$
$$H_2O_2 + 2H^+ + 2e \rightleftharpoons 2H_2O \qquad E° = +1{\cdot}35 \text{ volts}$$
$$O_2 + 4H^+ + 4e \rightleftharpoons 2H_2O \qquad E° = +0{\cdot}80 \text{ volts}$$

Fig. 5. Electronic configuration in relation to bond parameters for redox species of dioxygen. (Redox potentials from James, B. R. (1978) in *The Porphyrins* (Dolphin, D., ed.), p. 209. Academic Press, New York and London.

	Designation	Energy (kJ mol^{-1})	Electronic configuration		$t_{\frac{1}{2}}$ (s) in condensed phase
Second excited singlet	$^1\Sigma_g^+$	157	↑↓	↑↓	$\sim 10^{-9}$
First excited singlet	$^1\Delta_g$	94	↑↓	—	$\sim 10^{-6}$–10^{-4}
Ground state triplet	$^3\Sigma_g^-$	0	↑	↑	∞

Fig. 6. Electronic configuration and some properties of dioxygen and its first two excited states.

to be limited to the lower energy species (designated $^1\Delta_g$). For the purposes of this account, the term singlet oxygen will refer to this.

C. SOLUBILITY

The solubility of oxygen in biological fluids is of considerable importance. As Table 2 shows, the solubility of oxygen in water is low. Although there are a few examples of sluggish vertebrates, such as the Antarctic ice fish,[6] which manage without haemoglobin, the vast majority of higher animals employ such a haemoprotein as an oxygen carrier (Section III.B). The uptake of oxygen is thereby increased by an order of magnitude: arterial blood contains about 200 ml l^{-1} of oxygen.

In general, oxygen is more soluble in organic solvents than it is in water. This is especially so for fluorinated solvents, such as perfluorotributylamine, which indeed can function as a short-term substitute for blood in experimental animals.[7]

TABLE 2

Solubility of oxygen in various solvents†

Solvent	Temperature (°C)	Bunsen absorption coefficient‡
H_2O	20	0·0310
	35	0·0244
PhH	19	0·163
$CHCl_3$	16	0·205
MeOH	20	0·237
Et_2O	20	0·415

† From Landholt-Börnstein *Physikalisch-Chemische Tabellen* Springer, Berlin (1923), p. 765 and (1931) 2nd Supplement, Part-1, p. 484.

‡ Volume of oxygen (reduced to STP) which dissolves at the stated temperature in one volume of the solvent when the partial pressure of the gas is 760 mm.

D. REACTIVITY AND ACTIVATION

Oxygen is a rather unreactive molecule. This is partly due to the fact that the addition of one electron to give superoxide is endothermic. However, from that point on the reactions are energetically favourable. In thermodynamic terms the reaction of C–H bonds with oxygen is more favourable than is the reaction with chlorine. Living things, if they came into equilibrium with the earth's atmosphere, would be transformed principally into carbon dioxide and water. This does not happen because oxygen is much less reactive than is chlorine. The low reactivity of oxygen is associated with a high energy of activation: but, given an activating match to start if off, the forest will burn down.

Why is dioxygen so unreactive? One explanation that has been advanced has to do with spin state.[8] As discussed, dioxygen has a triplet ground state (Section II.B). According to one of the basic laws of chemistry, total spin is normally conserved during a physical or chemical process. Hence a reaction of the type:

$$\begin{array}{ccccc} \text{singlet} & + & \text{triplet} & \longrightarrow & \text{singlet products} \\ \uparrow\downarrow & & \uparrow\uparrow & & \downarrow\uparrow\ \downarrow\uparrow \\ S=0 & & S=1 & & S=0 \end{array}$$

which gives ordinary diamagnetic product molecules is formally forbidden. In practice this means that it will be less likely to occur than might have been expected.

How might dioxygen be made more reactive? Clearly, if the spin restriction could be removed, the reaction would be a more favourable process. In other

words, the dioxygen would appear to be activated. Although the argument is simplistic, it is worth continuing to see where it leads. Three principal ways in which the spin restriction could be lifted will be considered, namely:

(1) routes via free radical intermediates;
(2) routes via singlet oxygen;
(3) routes employing transition metal catalysts.

(1) *Routes via free radical intermediates*

The two pathways

$$\underset{\text{doublet}}{R^{\cdot}} + \underset{\text{triplet}}{O_2} \longrightarrow \underset{\text{doublet}}{ROO^{\cdot}} \rightleftharpoons \text{products}$$

and

$$\underset{\text{singlet}}{XH} + \underset{\text{triplet}}{O_2} \longrightarrow \underset{\text{doublet}}{XH^{+\cdot}} + \underset{\text{doublet}}{O_2^{-\cdot}}$$

are both spin-allowed processes. The first route is the generally accepted view of the mechanism by which autoxidation occurs, for example in unsaturated fatty acids and esters. Thus:

Initiation: Step 1.

$$\underset{\text{substrate}}{R^1{-}CH_2CH{=}CHR^2} + \underset{\text{initiating radical}}{R^{\cdot}} \longrightarrow RH + R^1\dot{C}HCH{=}CHR^2 \longleftrightarrow R^1CH{=}CH{-}\dot{C}HR^2$$

Propagation: Step 2.

$$R^1\dot{C}HCH{=}CHR^2 + O_2 \longrightarrow R^1\underset{\displaystyle OO^{\cdot}}{\underset{|}{C}}HCH{=}CHR^2$$

Step 3.

$$R^1\underset{\displaystyle OO^{\cdot}}{\underset{|}{C}}HCH{=}CHR^2 + \underset{\text{substrate}}{R^1CH_2CH{=}CHR^2} \longrightarrow R^1\underset{\displaystyle OOH}{\underset{|}{C}}HCH{=}CHR^2 + \underset{\text{propagating radical}}{R^1\dot{C}HCH{=}CHR^2}$$