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MOLECULAR STRUCTURE OF FREE RADICALS AND THEIR IMPORTANCE IN BIOLOGICAL REACTIONS

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Although inorganic chemists have been aware of free radicals for many years [21] it is only recently that their importance in biochemistry and medicine has become more widely appreciated. The toxic nature of free radicals was firmly brought to the attention of the public by the recent Spanish cooking oil disaster. More than 100 people died and 20000 were injured following the ingestion of oil which, although sold as olive oil, was in fact rapeseed oil which had been "refined" to remove the aniline and acetanilide (added to imported rapeseed oil for industrial use only). During this treatment toxins had been generated which were capable of inducing free radical-mediated damage [51].

The purpose of this brief review is to provide anaesthetists with a simple introduction to the chemistry of free radicals and a reference to fundamental information which may not be readily available in a medical library. Although the emphasis is on oxygen, general considerations apply to a wide range of free radicals and it is certain that, with the continuing research in this field, the crucial role of these chemical species in normal and pathological biochemical processes will become increasingly apparent.

An awareness of the chemistry of free radicals, and the way in which damage to cells and tissues occurs, is crucial to the understanding of certain disease processes and their control by free radical scavenging drugs. Examples include the various manifestations of oxygen toxicity, a variety of inflammatory processes, reperfusion injury, cancer and the ageing process. Such a knowledge is particularly important for the anaesthetist who routinely uses agents known to be associated with the generation of free radicals (oxygen and halogenated hydrocarbons). For such an understanding it is first necessary to examine in some detail the covalent bonding between atoms and the effect of single electron transfer reactions. Examples of free radical reactions in biological systems will be given, with particular emphasis on reactions of interest to the anaesthetist.

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Chemical bonding

In essence two types of chemical bond exist, ionic and covalent. *Ionic* bonding occurs when electropositive elements (for example, those from groups I and II of the periodic table, which lose their outermost electrons easily) combine with electronegative elements (for example, groups VI and VII elements, which tend to accept electrons easily). This interchange of electrons results in atoms gaining the most stable electron configuration—that is, that of the nearest inert gas (for example: $Na^+ \rightarrow Ne$, $Cl^- \rightarrow Ar$). Once formed, ions are held together by the electrical attraction of their opposite charge.

Covalent bonding requires the sharing of electrons between atoms. Atoms covalently bond because the potential energy of the molecule is lower than that of the atoms when they are present as separate entities. If sufficient energy is added to such a molecule the energy can be increased above that of the two separate atoms and the molecule will then dissociate (known as the bond dissociation energy). Either single (for example H_2), double (for example O_2) or triple (for example N_2) bonds can exist where the two atoms share 2, 4 or 6 electrons, respectively.

A single covalent bonding involving the sharing of electrons between two atoms (or molecules) A and B (signified either by: or - (A:B or A-B)) can break in three different ways:

$$A{:}B \rightarrow A{:}^- + B^+ \text{ heterolysis} \quad (1.)$$

$$A{:}B \rightarrow A^+ + B{:}^- \text{ heterolysis} \quad (2.)$$

$$A{:}B \rightarrow A^{\cdot} + B^{\cdot} \text{ homolysis} \quad (3.)$$

In (1.) and (2.), one of the atoms takes both electrons, resulting in the formation of ions. Fission of a single bond in this manner is known as heterolysis. Alternatively, each atom can take one electron, as in (3.). Known as homolysis, this reaction results in the generation of molecules, atoms or ions with an unpaired electron which are called free radicals (identified by $^{\cdot}$). In general, because of their unpaired electron status, free radicals are extremely reactive species, reacting rapidly with the majority of organic molecules which are resistant to the action of ions [41].

Free radical reactions

The main biological reactions in which free radicals participate are as follows:

$A^{\cdot} + B^{\cdot}$	$\rightarrow$ A-B	combination	(4.)
$A^{\cdot}$ + B-C-D$^{\cdot}$	$\rightarrow$ A-B + C=D	disproportionation	(5.)
A-B$^{\cdot}$	$\rightarrow A^{\cdot}$ + B	fragmentation	(6.)
$A^{\cdot}$ + B-C	$\rightarrow$ A-B + C$^{\cdot}$	radical transfer	(7.)
$A^{\cdot}$ + B=C	$\rightarrow$ A-B-C$^{\cdot}$	addition	(8.)

Generally, free radicals, particularly when formed in solution, react rapidly with many neighbouring molecular species. The radical half-life is usually very short and steady-state concentration is, therefore, low. However, in many biological systems, radicals are being continuously produced (for example, during oxidative phosphorylation).

There are, however, exceptions and some free radicals are more stable and, consequently, have longer half-lives. Indeed, free radicals were first identified because of a long-lived radical—the triphenylmethyl radical. Moreover, it is possible to keep otherwise unstable radicals for long periods of time by varying the conditions of storage such as pH and temperature.

Radical–radical reactions, (4.) and (5.), are extremely fast, but the rate depends on the square of the concentrations. Normally radical concentrations are extremely low so that reactions involving radical transfer (7.) and addition (8.) predominate. These reactions usually involve a chain process passing through initiation, propagation and termination stages. It is this process of radical propagation that is responsible for most of the damaging effects of free radicals (for example lipid peroxidation, described below).

The main biological targets of free radical attack are lipids, sulphydryl-containing proteins and DNA. Figure 1 shows the initiation of peroxidation in polyunsaturated fatty acids as a result of attack by reactive species resulting in a weakening of double bonds. Molecular rearrangement then occurs, forming a conjugated diene (-C=C-C=C-) which readily reacts with oxygen to form a peroxy radical (R-OO$^{\cdot}$). These radicals can cause hydrogen abstraction from another lipid molecule, with consequent weakening of the double bonds, resulting in propagation of the peroxidative process by a chain reaction. Membranes, being largely composed of unsaturated lipid and protein, are thus particularly vulnerable to oxidative attack. This process is familiar as it results in the rancidification of fat.

Proteins are also damaged by free radical reactions. It has been known for some time that many enzymes are rapidly inactivated by hyperoxia *in vitro* [25]. A general characteristic of such enzymes is their content of sulphydryl groups (-SH). These are easily oxidized, by molecular oxygen, free radicals or peroxides [29]. Conformational changes are then induced in the enzyme, resulting in decreased activity.

An example of the changes which occur in sulphydryl-containing compounds is shown where oxidation by the free radical $A^{\cdot}$ results in an intermediate thiyl radical which can then interact to form sulphur–sulphur bonds. Such a reaction is thought to explain the protective effect of thiol-containing compounds (such as cysteamine, methionine and n-acetylcysteine) in radiation injury, paracetamol poisoning and attack by radicals derived from oxygen, such as superoxide anion.

$$\begin{aligned} R\text{-}SH + A^{\cdot} &\rightarrow R\text{-}S^{\cdot} + AH \\ &+ \\ R\text{-}S^{\cdot} &\rightarrow R\text{-}S\text{-}S\text{-}R \end{aligned}$$

Gluthathione is a sulphydryl-containing tripeptide which can be recycled by the interaction of the oxidized form with NADPH. This recycling of glutathione makes it particularly effective as a free radical scavenger.

$$R\text{-}S\text{-}S\text{-}R + NADPH + H^{+} \rightarrow 2R\text{-}SH + NADP^{+}$$

Free radical damage to nucleic acids and nucleotides has been extensively studied with particular reference to the effects of ionizing radiation and carcinogenesis [19, 30, 31]. It would seem that most destruction occurs by radical attack of the pyrimidine to deoxyribose bond,

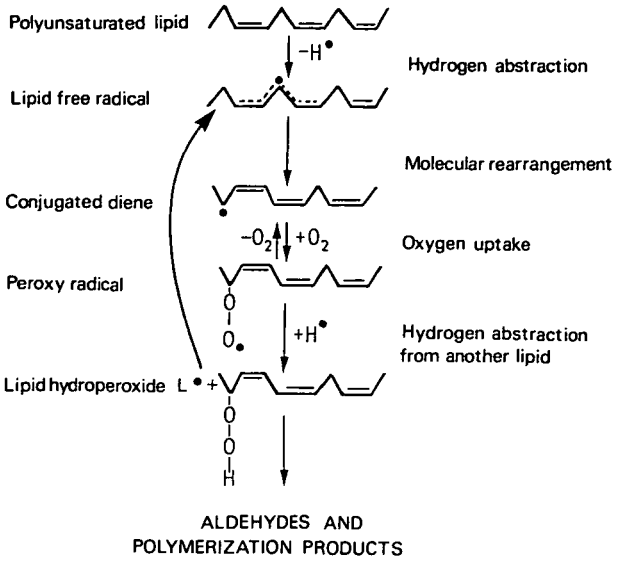

FIG. 1. Initiation and propagation reactions of lipid peroxidation.

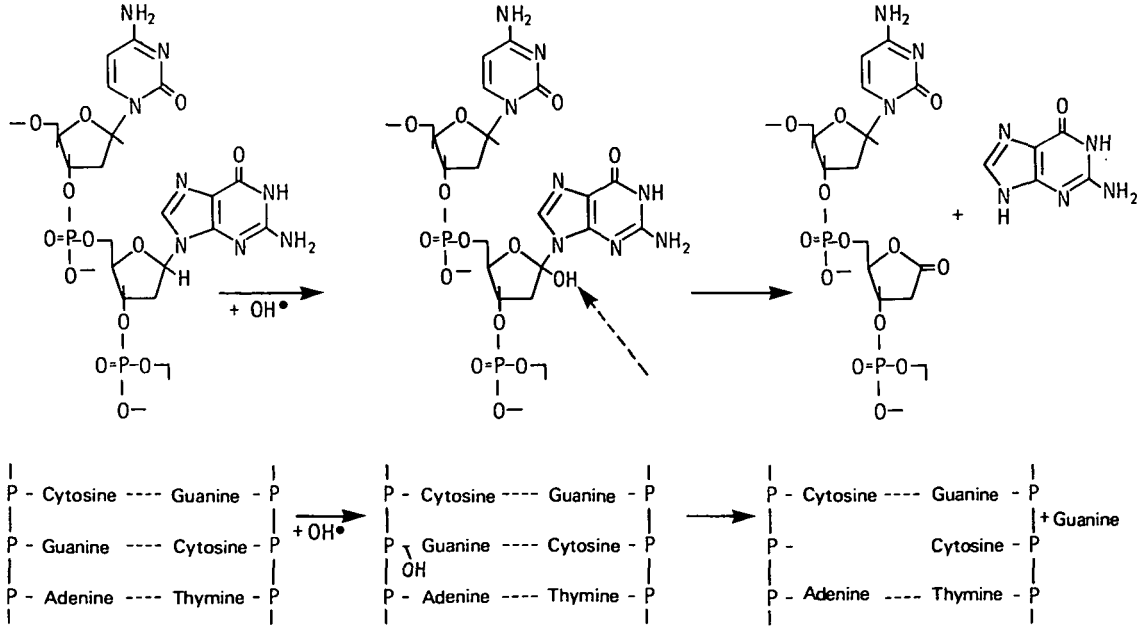

FIG. 2. One possible mechanism for hydroxyl radical-induced single strand scission of DNA. The lower figure illustrates the effect that this would have on the double strand DNA.

with breakage of the sugar–phosphate bond and liberation of free bases from the nucleotides (fig. 2).

It is now thought that the reactivity of a radical in an isolated chemical system is a relatively unimportant predictor of the degree of biological damage that may result from the exposure of the intact organism to that radical. An unstable radical will be more likely to interact with itself and any other molecule it meets, whereas a more stable

radical may produce more damage by reacting only with certain, and perhaps crucial, chemical species. It is believed that these latter sites of interaction are molecules of major biological significance (for example, the amino acids which determine the conformational structure of proteins).

Protective systems

To prevent damage arising from excess free radical concentrations, a variety of protective mechanisms are used. These essentially fall into two groups:

(1) *Preventative or primary antioxidants*. These reduce the rate of initiation of free radical chain reactions by decreasing the concentration of free radicals available to react with cell components.

(2) *Chain-breaking or secondary antioxidants*. These trap the chain propagating radicals, thus arresting the destructive chain reactions at an early stage.

There are five groups of free radical scavenging agents which can be used in biological systems:

(1) Water soluble compounds, for example ascorbic acid and the sulphydryl containing glutathione (γ-glutamyl-cysteinyl-glycine).

(2) Naturally occurring fat soluble compounds, for example α-tocopherol and β-carotene.

(3) Substances bound to high molecular weight materials, for example the sulphydryl-containing proteins.

(4) Exogenous compounds, for example the food antioxidants (butylated hydroxytoluene, propyl-gallate, ethoxyquin and nordihydrogaurietic acid) and drugs such as the phenothiazines.

(5) Enzymes. There are three enzymes intimately concerned with the detoxification of oxygen-derived free radicals:

Superoxide dismutase catalyses the reaction of two superoxide anions ($O_2^{\cdot -}$) with the consequent formation of hydrogen peroxide:

$$O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2$$

Two enzymes guard against damage from hydrogen peroxide: catalase and glutathione peroxidase. *Catalase* is highly specific and has appreciable activity only for hydrogen, methyl and ethyl hydroperoxides:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Glutathione peroxidase has a more general action and catalyses the reduction of many hydroperoxides (R-OOH) by reduced glutathione (G-SH):

$$R\text{-}OOH + 2G\text{-}SH \rightarrow R\text{-}OH + G\text{-}S\text{-}S\text{-}G + H_2O$$

(Note that R-OOH is different from an organic acid R-COOH such as acetic acid CH_3-COOH.)

Although deficiency of either catalase or glutathione peroxidase has been reported in man, only anaerobic organisms and a very few aerobic bacteria are without superoxide dismutase.

Oxygen and oxygen-derived free radicals

Molecular oxygen (dioxygen) is a stable biradical, as it has an unpaired electron in the outer shell of each constituent oxygen atom. This may at first seem somewhat surprising, as the total number of electrons in molecular oxygen (16) would suggest that each is fully paired. However, a more detailed examination of molecular electron configuration (given in the Appendix) will reveal that this cannot be so. Moreover, largely as a result of the constraints imposed by energy conservation, the two unpaired electrons of molecular oxygen spin in the same direction (parallel spin). It is this latter fact that explains the paramagnetic properties of molecular ground state oxygen. Moreover, it also prevents it acting as a free radical since, if these electrons spin in opposite directions (as in singlet oxygen), a much more reactive molecule results.

Oxygen readily accepts electrons from other molecules; that is, it is a powerful oxidizing agent, and each molecule of oxygen can accept a total of two pairs of electrons. However, pairs of electrons cannot be accepted at the same time, since both of these electrons would have to be in parallel spin to fit into the free electron orbitals. Such a pair of electrons cannot exist, because any pair must necessarily have opposite spins in accordance with basic chemical principles. The so-called spin restriction forces oxygen to accept only one electron at a time and means that oxygen reacts only slowly with many non-radical compounds.

More reactive forms of oxygen can, however, be generated. With the input of energy, the two parallel spinning electrons can take up antiparallel spins with the formation of two possible forms of what is known as singlet oxygen, $^1\Delta gO_2$ (energy level of 22.4 kcal above ground state) or $^1\Sigma g^+$ (37.5 kcal above ground state). In both of these singlet forms spin restriction has been removed and the oxidizing ability of oxygen is greatly

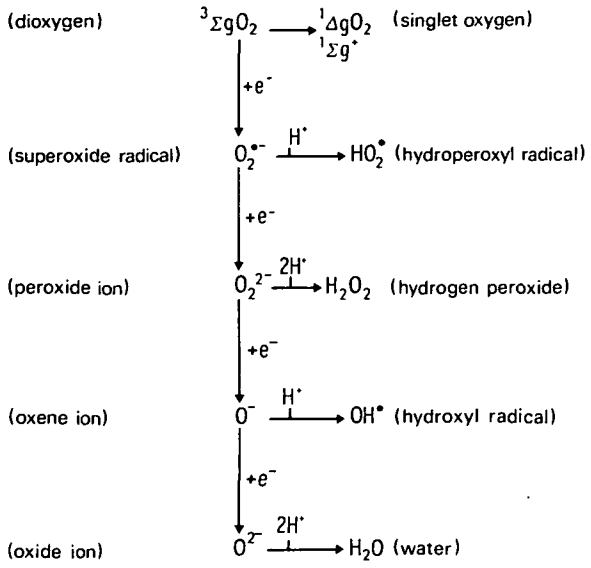

FIG. 3. Products derived from the successive one electron reduction of dioxygen.

increased. Alternatively, electrons can be added to ground state molecular oxygen singly (fig. 3).

The addition of an electron to ground state dioxygen (giving what is known as the one electron reduction product) forms the free radical superoxide anion ($O_2^{\cdot-}$). In biological systems the two-electron reduction product of ground state oxygen is hydrogen peroxide and the four-electron product is water. This reaction is occurring continuously within the cell during oxidative phosphorylation. Some oxidases, such as cytochrome oxidase, reduce oxygen to water without detectable formation of free radical intermediates, apparently producing a one step four-electron reduction of oxygen to water [10]. However, it is thought that oxygen derived radicals are formed, but are normally bound to active sites within the enzyme and, therefore, remain "hidden" from other intracellular components [7].

Although ground state dioxygen is an oxidizing agent (it accepts electrons readily), superoxide anion mainly undergoes reducing reactions (it readily donates an electron).

Hydroxyl radical and hydrogen peroxide

It would seem unlikely that $O_2^{\cdot-}$ is responsible for all the damage seen in experimental systems involving oxygen-derived free radical species, and it is well known that hydroxyl radical ($OH^\cdot$) is much more reactive than $O_2^{\cdot-}$. Reactions which produce $OH^\cdot$ do occur in cells. One such reaction (known as the Haber–Weiss reaction), which was first described in a purely chemical system, involves the reaction of hydrogen peroxide with $O_2^{\cdot-}$:

$$H_2O_2 + O_2^{\cdot-} \rightarrow O_2 + OH^- + OH^\cdot$$

Because of the low reaction rate constant and the small steady state $O_2^{\cdot-}$ concentration, this reaction is unlikely to occur *in vivo* [24, 36]. However, the rate constant can be greatly increased by trace concentrations of transition metals (for example copper and iron). This is known to occur *in vivo* (the Fenton reaction) and, similarly, involves transition metals and hydrogen peroxide thus:

$$Fe^{3+} + O_2^{\cdot-} \rightarrow Fe^{2+} + O_2$$
$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^\cdot + OH^-$$

The importance of transition metals in free radical generation within biological systems has only recently been appreciated and would suggest that chelators, which bind such metals and render them inactive as catalysts, may be useful therapeutic agents in some disease processes.

Irradiation and the oxygen effect

The effects of ionizing radiation in an aqueous system are shown in reactions 1–4 below. The subsequent interactions with oxygen and the products $H^\cdot$ and e^- (reactions 5–7) result in an enhancement of the effects of irradiation at high partial pressures of oxygen [20, 22]. This principle has been used in tumour radiotherapy.

1. $H_2O \rightarrow H_2O^+ + e^-$
2. $H_2O + e^- \rightarrow H_2O^-$
3. $H_2O^+ \rightarrow H^+ + OH^\cdot$
4. $H_2O^- \rightarrow H^\cdot + OH^-$
5. $O_2 + H^\cdot \rightarrow HO_2^\cdot$
6. $O_2 + e^- \rightarrow O_2^{\cdot-}$
7. $O_2^{\cdot-} + H^+ \rightarrow HO_2^\cdot$
8. $2HO_2^\cdot \rightarrow H_2O_2 + O_2$

Oxygen toxicity and irradiation are in many ways similar and Gerschman and colleagues [18] were the first to propose that oxygen-derived free radicals are responsible for the pathophysiology of oxygen poisoning. This suggestion has led to the investigation of many radioprotective substances as possible therapeutic agents for oxygen toxicity

[11, 28, 58], but as yet no agent has been found to be of particular benefit.

Ozone and the oxides of nitrogen

Other important and powerful oxidants are ozone and nitrogen dioxide, both common environmental pollutants. The molecular structure of ozone is such that the O–O bonds (bond distance 1.26 Å) are intermediate in character between single and double bonds as the bond distance of HO-OH (single bond) is 1.49 Å and that of oxygen O,O (double bond) is 1.10 Å [44]. Tetratomic oxygen (O_4) also exists, but is present in appreciable quantities only in liquid and solid oxygen [32]. Little is known about the reactivity of this molecule, which is made up of two O_2 molecules held together by forces which are intermediate between electron sharing and Van der Waals electrostatic bonds.

Nitric oxide and nitrogen dioxide are free radicals, and are capable of directly causing lipid peroxidation by attacking unsaturated carbon bonds. Nitric oxide reacts with oxygen to form nitrogen dioxide and both oxides can react with hydrogen peroxide (readily available *in vivo*) to produce hydroxyl radicals. The higher oxides of nitrogen may contaminate nitrous oxide used in anaesthesia and some of their effects are attributable to free radicals [38, 50]. Radiation chemists routinely generate hydroxyl radical by the reduction of nitrous oxide by aqueous electrons from gamma irradiation:

$$N_2O + e^- \rightarrow OH^{\cdot} + OH^- + N_2$$

Another major environmental pollutant is smoke, including cigarette smoke. Inactivation of alpha-1-antiproteinase by the oxidants in smoke diminishes the antiproteinase defenses of the lung and is suggested to be the method by which excess protease activity results in the pathogenesis of emphysema [8]. In a similar manner, oxidants released from stimulated leucocytes could result in inactivation of antiproteinases.

Bipyridyl herbicides and paracetamol

These commonly ingested poisons are thought to produce damage by free radical intermediates. The bipyridyl compounds paraquat and diquat easily cross membranes and readily accept electrons from the electron transport chain of either the chloroplast or the mitochondrion, with the formation of the bipyridyl radical ($BP^{\cdot+}$). In the presence of oxygen, superoxide anion is rapidly formed, with the consequent production of hydrogen peroxide and hydroxyl radical [47]:

$$BP^{2+} + e^- \rightarrow BP^{\cdot+}$$

$$BP^{\cdot+} + O_2 \rightarrow BP^{2+} + O_2^{\cdot-} \text{ (Note } BP^{2+} \text{ is recycled).}$$

The type I pneumocyte of the lung is unfortunate in both accumulating paraquat or diquat against a concentration gradient as well as having the greatest Po_2 in the body.

In similar fashion paracetamol is metabolized via a free radical intermediate which is capable of causing direct cell damage [15].

Halogenated hydrocarbons

Carbon tetrachloride (CCl_4), chloroform ($CHCl_3$) and halothane ($CF_3CHBrCl$) are halogenated hydrocarbons which form free radical intermediates during biotransformation. When they are metabolized by the cytochrome P450 system homolytic fission occurs, with the generation of free radicals which then undergo chain propagation reactions with other anaesthetic molecules, lipids, thiols or oxygen.

$$CCl_4 \rightarrow Cl^{\cdot} + CCl_3^{\cdot} \rightarrow CCl_3O_2^{\cdot}$$

$$CHCl_3 \rightarrow H^{\cdot} + CCl_3^{\cdot}$$

$$CF_3CHBrCl \rightarrow Br^{\cdot} + CF_3CHCl^{\cdot}$$

Alternatively, an electron may be added to the reaction from the mixed function oxidase P450 system:

$$CF_3CHBrCl + e^- \rightarrow Br^- + CF_3CHCl^{\cdot}$$

These changes have been particularly studied with carbon tetrachloride and halothane, which are both capable of causing centrilobular hepatic necrosis in certain animal models. It is probably the mechanism which results in the enhanced hepatotoxicity seen with many of these compounds following stimulation of P450 activity with phenobarbitone [33, 39, 40, 45, 55].

Oxygen toxicity

Although oxygen therapy is clearly beneficial in many clinical settings, it has long been recognized that it also carries a risk of tissue damage. This action was first demonstrated by Paul Bert [5] using hyperbaric oxygen and was later found also at normobaric pressures by Smith [46].

The lung is exposed directly to the highest partial pressures of oxygen and atelectasis, oedema, alveolar haemorrhage, fibrin deposition

and thickening of alveolar membranes can be demonstrated in animals following exposure to 80–100% oxygen for 2 days. The cells most clearly damaged by oxygen are the capillary endothelium and the alveolar type I epithelial cells.

Currently, the most popular theory to explain the pathology of oxygen toxicity is that specific damage occurs as a result of the intracellular production of oxygen-derived free radicals in excessive concentrations which overwhelm the natural defences of the cell. Support for this theory comes from two sources. First, it has been demonstrated that mitochondria release excess superoxide anion under hyperoxic conditions [56]. Second, it is possible to produce enhanced endogenous antioxidant activity by treatment of rats with 85% oxygen; these animals will then tolerate exposure to 100% oxygen for prolonged periods [12].

Retrolental fibroplasia

This condition, which can lead to blindness, is believed to be a complication of the use of an increased partial pressure of oxygen in premature infants [43]. There are two possible aetiologies for this disease: vasoconstriction resulting in ischaemia, and increases in the concentrations of oxygen-derived free radicals. In addition to the increased partial pressure of oxygen within the eye as a result of oxygen therapy, singlet oxygen is generated as a result of u.v. irradiation. However, retrolental fibroplasia still occurs, even with the closely monitored administration of oxygen, and it is now considered that "excessive" oxygen is only one factor in what is a disease with a multifactorial aetiology [16, 34]. Nevertheless, there is evidence that the administration of tocopherol may reduce the risk of developing severe retrolental fibroplasia [26].

Reperfusion injury

It is now becoming apparent that free radicals may play some role in the pathology of ischaemia and reperfusion injury [35]. It has long been accepted that the major adverse changes seen in ischaemia are caused by the depletion of adenosine triphosphate (ATP) and an increase in H^+ during the period of hypoxia. However, another possible explanation is that the deleterious effects actually occur during reperfusion and are the result of the generation of free radicals from dioxygen as a result of oxidation of the accumulated reduced compounds [17, 42] (fig. 4). Xanthine oxidase reduces oxygen to $O_2^{\cdot -}$ which is then released into the intra- and extracellular fluid. Xanthine oxidase is thought to be generated from xanthine dehydrogenase (an enzyme which transfers electrons from substrates on to NAD^+ rather than O_2) during periods of ischaemia, presumably by the action of proteolytic enzymes [4]. There is also an increased concentration of hypoxanthine (another substrate of xanthine oxidase) during ischaemia (fig. 4). Allopurinol, a xanthine oxidase inhibitor, has been shown to prevent ischaemic damage [42].

Currently, there is much interest in the possible use of antioxidants and free radical scavengers in the prevention of reperfusion injury during cardiopulmonary bypass, in an attempt to improve myocardial function following arrest. Protection of mechanical and subcellular function has been reported by Stewart and co-workers [48] using a cardioplegic solution of superoxide dismutase, mannitol and potassium chloride in dogs on cardiopulmonary bypass, while other workers have found significant improvement in patients undergoing bypass when a single oral dose of vitamin E (2000 iu) was given 12 h before operation [9].

Another area where free radicals generated as a result of ischaemia are important is in the field of transplantation, where much current research is directed at limiting the effects of free radical-induced damage by treatment before and after the period of organ ischaemia [49].

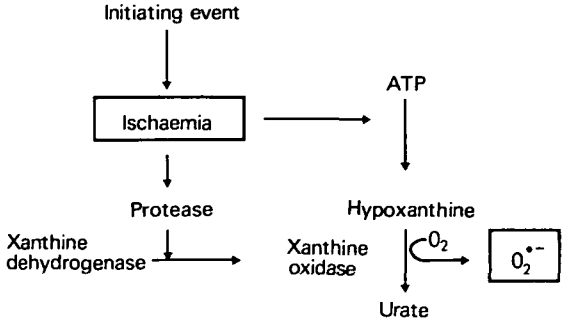

FIG. 4. The generation of superoxide anion ($O_2^{\cdot -}$) resulting from ischaemia and reperfusion. Ischaemia permits the conversion of xanthine dehydrogenase to the oxidase form, and provides an excess of substrate (purine metabolites) for this enzyme. Reperfusion and reoxygenation then allows the xanthine oxidase reaction (which requires oxygen) to occur with production of superoxide anion.

ARDS and multiple organ failure

Adult respiratory distress syndrome (ARDS) is a form of acute lung injury characterized by hypoxaemia refractory to treatment, together with a high permeability pulmonary oedema. The pathogenesis of ARDS is complex and involves, probably, many mechanisms including microthrombus formation [23], prostaglandin release [6] and intrapulmonary sequestration and stimulation of polymorphonuclear leucocytes [13]. As described below, stimulated white cells will release both oxygen-derived free radicals and connective tissue proteases. Large numbers of white cells have been identified in bronchoalveolar lavage fluid obtained from ARDS patients and this fluid has also been found to contain myeloperoxidase and collagenase (released from white cells) [57]. Further characteristics of ARDS, high pulmonary artery pressure and vascular resistance, can also be attributed to increased toxic oxygen metabolites. In an experiment on isolated perfused rabbit lungs Tate and colleagues [53] have shown that such metabolites stimulate thromboxane production, by enhancing the metabolism of arachidonic acid, and thus cause vasoconstriction.

Plasma lipid peroxide concentrations have been shown to be increased in critically-ill patients in Intensive Care Units, and this is associated with significant reductions in plasma alpha-tocopherol concentrations [52]. The highest lipid peroxide concentrations were found in septic patients and, in particular, those who developed disseminated intravascular coaggulation (DIC). The authors suggest that antioxidant therapy with, for example, alpha-tocopherol may prevent peroxidation in patients liable to develop DIC and multiple organ failure.

If antioxidants are to be used in conditions such as ARDS and multiple organ failure, then tissue-specific targeting may become important. In this regard it has been suggested that polyethylene glycol (PEG) conjugation with antioxidants may be more effective than liposome encapsulation [37]. Delivery of antioxidants conjugated to PEG has been shown to be improved and their half-lives prolonged over that with liposome encapsulation.

Biologically useful radical reactions

All reactions discussed so far have been deleterious; however, free radical reactions within cells are often useful, and some are essential for normal cell function. Once the polymorphonuclear leucocyte is attracted to sites of infection and stimulated to destroy the foreign materials, it undergoes a series of oxygen-dependent reactions with the release of superoxide radical [1, 2]. Individuals with chronic granulomatous disease lack the enzyme NADPH oxidase and, therefore, the capacity to generate superoxide radical. Such patients have an abnormal susceptibility to bacterial infections [3, 27]. It is possible that some inflammatory diseases (for example rheumatoid arthritis, adult respiratory distress syndrome and autoimmune disorders) are caused by the excessive or inappropriate stimulation of leucocytes, with the consequent release of high concentrations of superoxide radical [14, 54].

There are many other important reactions where free radical intermediates are essential for normal cell function, including the formation of deoxyribonucleotides from ribonucleosides, oxidation, carboxylation and hydroxylation reactions and prostaglandin metabolism.

CONCLUSIONS

With greater understanding of the role of free radical reactions in disease processes, it should be possible to investigate more rational approaches to therapy. Such therapy would be directed towards reducing free radical concentrations by either preventing their formation or preventing their reaction, by scavenging, with other molecules of importance within the cell.

APPENDIX

Structure of the atom

Electrons possess a negative charge and must, therefore, have energy to prevent them from being pulled into the positively charged nucleus. They exist in shells (1, 2, 3...7) and move around the nucleus in one of four orbital patterns, spinning in either direction. Each of these features (except for spin direction) is related to a particular energy level. The s orbital is spherical, but the others (p, d and f) can be described in one of three different planes (x, y and z) each with a maximum of two electrons around the nucleus. The p orbit, the only one to concern us here apart from s, is shown in figure 5.

The notion of an electron having a discrete position (for example following a definite orbit) has now been replaced by the Schrodinger concept of a probability distribution which describes how the density of the charge cloud is distributed in space. This concept allows the charge distribution to be calculated for atoms as well as molecules, where electrons occupy orbitals centred on both nuclei.

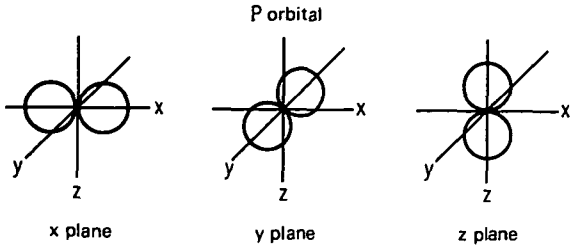

FIG. 5. The three planes of the p orbit electrons.

The electronic structure of any atom or molecule may be defined by taking account of the following factors:

(1) Orbitals are filled in order of increasing energy.

(2) Each orbital may hold only two electrons with opposite spins (the Pauli exclusion principle).

(3) Where several orbitals of equal energy are available (for example the 3p orbitals of shell 2) the electrons fill each singly, keeping their spins parallel, before spin pairing occurs. This is known as Hund's rules: as far as possible electrons avoid being in the same orbital, and electrons in different orbitals of the same energy have parallel spins.

Structure of the molecule

In the case of molecules, electrons can "interact" with each other either "constructively" or "destructively" and are said to be either in bonding orbitals where there is an accummulation of electron density in the region between the two nuclei (this has the effect of reducing their internuclear repulsion); or in antibonding orbitals (signified by *) where the probability of finding an electron between the nuclei is zero. In the absence of electrons between the nuclei, the internuclear repulsive force has full effect (fig. 6).

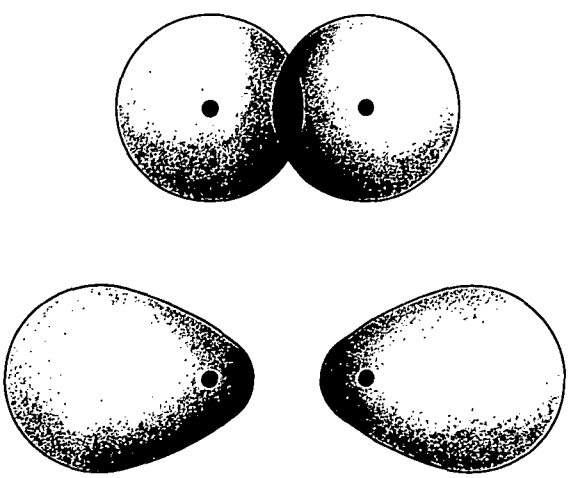

FIG. 6. Bonding and antibonding orbitals. In the upper figure the s electron cloud from each atom are interacting in such a way as to provide a high probability of finding a negative charge between the two nuclei. This reduces the internuclear repulsion to zero and the electrons are said to lie in a bonding orbital (σ). In the lower figure there is a zero probability of finding a negative charge between the two nuclei and the internuclear repulsive force is maximum; an antibonding orbital (σ*).

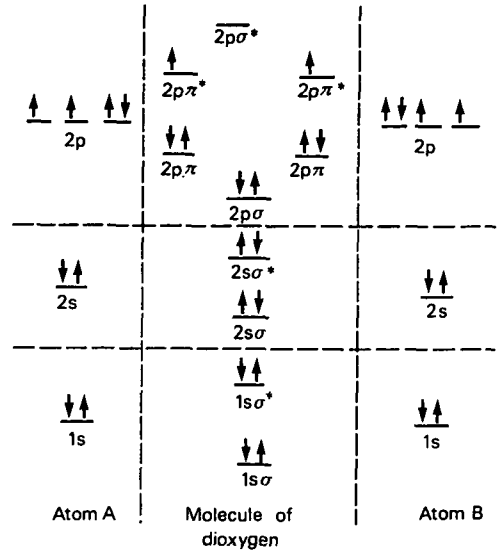

FIG. 7. The detailed electron structure of dioxygen. Two oxygen atoms (Atom A and Atom B) are shown on either side of the molecule of dioxygen which results from their combination.

The s atomic orbital electron density is symetrical and, in a molecule, two such electrons can interact to form molecular orbitals known as σ which may be either bonding (sσ) or antibonding (sσ*). Similarly, two p_z orbital electrons can interact to form molecular orbitals like those formed by s atomic orbitals and these are known as pσ or pσ*. The p_x or p_y atomic orbitals cannot enter into σ bonding, but can overlap sideways to form π bonds (again either bonding (pπ) or antibonding (pπ*)). Electrons enter each of these molecular orbitals in strict order of energy level, filling the lower levels before entering higher levels. Energy levels increase in the order $1s\sigma < 1s\sigma^* < 2s\sigma < 2s\sigma^* < 2p\sigma < 2p\pi < 2p\pi^* < 2p\sigma^*$.

Structure of the oxygen molecule

The oxygen atom has eight electrons distributed as follows: $1s^2 2s^2 2p^4$ (fig. 7). When two atoms join to form diatomic oxygen (dioxygen) the molecular electron distribution can be calculated from the rules given above. The four 1s electrons (two from each atom) fully occupy both 1s bonding and 1sσ* antibonding molecular orbitals. The four electrons in the next shell which have s atomic orbitals occupy 2sσ and 2sσ* molecular orbits. Eight outer electrons are now left; two exist in $2p_z$ orbitals making a 2pσ bonding orbit. The 2pσ* antibonding orbitals are not occupied because they are of higher energy level than both the pπ and pπ* molecular orbitals. The $2p_x$ and $2p_y$ atomic orbitals can overlap laterally to give $p_x\pi$ and $p_y\pi$ bonding molecular orbitals. The remaining two electrons must occupy the next highest molecular orbital in energy; there are two such orbitals $2p_x\pi^*$ and $2p_y\pi^*$, and by Hund's rule, each must receive one electron. Since the presence of one electron in each of these molecular orbitals cancels one of the 2pπ bonding orbitals, the two oxygen atoms

are effectively joined by a double bond. Molecular oxygen therefore, has two unpaired electrons in the outer shell occupying $2p_x\pi^*$ and $2p_y\pi^*$ orbitals (fig. 7).

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