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The Necessary and the Desirable Production of Radicals in Biology [and Discussion]

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The necessary and the desirable production of radicals in biology

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The production of radicals within biological systems can be necessary, as in the coupling of light capture or oxygen–hydride reactions to the generation of proton gradients in thylakoid and mitochondrial membranes; or it can be extremely desirable, to produce an effective catalyst centre in, for example, ribonucleotide reductases. To fit their function the radicals are usually of controlled reactivity, either through their redox properties or through confinement to a limited volume. The more indiscriminate generation of radicals can be of great use too in protective devices, for example, in the production of cross-linked polymers, or in the generation of oxygen radicals. The different distribution and release of initiating enzymes for all these radical processes ensures the spatial and temporal control of their reactions.

Introduction

This article is written to correct an impression that is growing in the biochemical community. Free radicals in biological systems are said to be dangerous and associated almost exclusively with the onset of diseased states such as cancer. To give a more balanced picture I shall deliberately look for those situations in which free radicals are essential for a particular biological system. There are two points of departure. First, free radicals are not necessarily extremely reactive. Oxygen itself, superoxide and flavin radicals *per se* are relatively stable and have either low thermodynamic reactivity or have considerable chemical kinetic barriers to reaction. The chemistry of free radicals is open to the same controls as that of non-free-radical chemistry. Secondly, even highly reactive free radicals can be generated in physically restricted parts of a biological system, where they may be extremely valuable. The spatial restrictions are dependent on the sites of production and thereafter on the prevention or limitation of diffusion of the radicals. By placing enzymes in special compartments, for example, extracellular rather than intracellular, by confining radical production to lipid bilayers through solubility restrictions, or by using radical intermediates in closed forms of hinged enzymes, the much discussed dangers inherent in radical reaction with DNA can be minimized and radicals can then be put to very effective use. If radicals had been so dangerous, surely during the process of evolution they would have been avoided; in fact they are used by all cells. The risks have always to be measured against the advantages in evolution, or elsewhere.

The redox potentials and reactivity of biological radicals

The simplest control over radical reactivity rests in the redox potential. Radicals in organic molecules are stabilized by conjugation so that in the reversible steps

$$X \rightleftharpoons X^{+\cdot} \rightleftharpoons X^{2+}$$

the radical may not be of such a high energy that disproportionation is grossly favoured. We see this in flavin and quinone chemistry where the two one-electron redox potentials of (1) in

table 1 are not greatly different. The reasons for the stability of such radicals can be discussed in terms of the nature of their π-electron systems, and radicals stabilized by such orbitals usually have relatively low redox potentials. On the whole, radicals in σ-antibonding or non-bonding (lone-pair) orbitals are much less stable, for example, in $RCH_2^{\bullet}$. Such radicals are inherently dangerous if they can diffuse freely. They usually have high redox potentials.

Table 1. Redox potentials of some radicals[a]

oxidizing reagent	successive one-electron redox potentials (volts)	
O_2	−0.2 (O_2^-),	−0.6 ($OH^{\bullet}$), then +2.0 (H_2O)
		+0.8 (H_2O_2), then +1.2 (H_2O)
quinone	−0.2 ($Q^{\bullet}$),	+0.3 (QH_2)
flavin$^+$	−0.4 ($F^{\bullet}$),	+0.2 (FH)
$[Fe^{IV}O]^{2+}$	*ca.* +1.00 (Fe^{III}),	−0.3 (Fe^{II}) in peroxidases
$[Mo^{VI}O_2]^{2+}$	−0.3 (Mo^{V}),	−0.3 (Mo^{IV})
dehydro ascorbate	−0.2 ($A^{\bullet}$),	+0.3 (AH_2)
disulphides (glutathione)	−0.3 ($RS^{\bullet}$),	+0.1 (RSH)
simple phenol radicals		*ca.* +1.00 (phenol)
simple indole radicals		*ca.* +1.00 (indole)

[a] The potentials are the one-electron redox potentials required to go from the species on the left to the species on the right in each case, and a positive sign indicates a powerful oxidizing action. Measured at pH 7 and 25 °C. The redox potentials of all the coenzymes are adjusted within their enzymes to meet requirements of specific reactions.

Inorganic free radicals are not so readily described because we must distinguish light-atom free radicals, such as $NO^{\bullet}$, from metal-ion radicals. It is not conventional to call Cu^{II}, Fe^{III}, Mn^{II} or Mo^{V} free radicals although they do have unpaired electrons in d-shells. This is unfortunate in the context of this article, because much of the organic free-radical production in biology starts from reactions of these (free-radical) transition metal ions. Metal ions can be made into extremely reactive free radicals by exposing or elevating the energies of the unpaired d-electrons. Thus low-spin Fe^{III}, a 3d free radical, is often very reactive as compared with the high-spin Fe^{III} ion. Passing to elements of the second or third transition metal series the unpaired electrons become relatively more reactive as, for example, in Mo^{V}. We know that this is not a matter of redox potential but of the *exposure* of the electron, which in all cases is in screened d-orbitals, but is more exposed the higher the d-shell number. The extreme case of the hiding of unpaired electrons is shown in the 4f-core of the lanthanide cations, which do not behave as free radicals. Biological molecules can hide organic free electrons within complex molecules, proteins, as successfully as this hiding of free d-electrons in the core of metal ions. We see that it is the mixture of thermodynamic stability, local kinetic reactivity, and physical protection that allows both one-electron reactions of inorganic and organic chemistry to be controlled. Biology has learned to combine the inorganic and organic free-radical chemistry within such control structures to make extremely valuable devices. Sir Derek Barton (this symposium) introduces many of these ideas with respect to the not-so-obvious case of the B-class elements of the Periodic Table.

Radicals in extracellular space

When remote from the cell nucleus a radical is no more dangerous than a nucleophile or an electrophile. It is just a potential intermediate in a reaction. Some reactions need radicals and in such cases biology must use them. A clear example is free-radical polymerization, which

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gives a plastic. Thin layers of plastics are common devices in the surface structures of plants and animals, especially insects, and they are found in the skin of mammals in the form of melanin pigments. The initial radical polymerization is induced in response to growth hormones, as in insects, or in response to damage by sunlight or physical injury as when a potato or apple is cut. The reactions use a vesicular store of phenols or indoles related to tyrosine and tryptophan as the source of monomer. The exposure of these aromatic molecules to one-electron oxidation by oxygen or hydrogen peroxide in association with metallo-enzymes is the initiation step, and because many of these molecules are bifunctional they allow cross-linking as well as engaging in simple chain propagation. A firm plastic material such as insect cuticle, is generated. Thus the skeleton of insects and of many other animals, for example, the crab, is thus effectively a result of controlled free-radical reactions rather than mineralization. Many pigments are made this way too. We can look at the controls which make this possible.

The oxidizing agents O_2 and H_2O_2 diffuse freely across membrane barriers, but both reagents need metal catalysts to induce reaction. It is then the positioning of these catalysts and organic molecules that is the essential feature of radical production. The enzymes (metallo-enzymes) and the organic monomer substrates must be placed in separate compartments, only to be brought together when required. Some of these compartments are known (table 2). As far as the catalysts are concerned, most are copper proteins that are confined to extracellular fluids by their synthesis. It is a feature of biology that copper is very rarely found intracellularly.

Table 2. Examples of compartments of free-radical generation and reaction

reagent	catalyst
O_2 freely diffusing	extracellular phenol oxidases (Cu)
	extracellular ascorbate oxidase (Cu)
	extracellular caeruloplasmin (Cu)
H_2O_2 freely diffusing	peroxisomes, peroxidases (Fe)
light	components of melanin granule
oxygen and the superoxide	phagosomes (flavin enzymes)

(Superoxide dismutases are a very special case and are very well designed enzymes for a very particular purpose in the cytoplasm of cells as we shall see.) The monomers are then released into this extracellular space. The extracellar cross-linking activity extends from phenol oxidases and laccases to lysine oxidases and is important in cross-linking collagens for the production of bone as well as for the synthesis of radical polymers (Williams 1982).

Another type of catalyst is the iron enzymes, which are rarely if ever found free in extracellular fluids. Most iron enzymes are intracellular but the ones of interest here are held in vesicles, for example, peroxidases in peroxisomes and are only released from the vesicles in response to cell damage. The monomers of the reaction are stored in such bodies as melanocytes quite separately from these peroxidases. Once again, it is the release from special compartments into a particular common space that makes for radical polymerization in a controlled way.

There is another possible use for free radicals in extracellular space, and that is in the scavenging of other free radicals that have escaped or are potentially dangerous. A dangerous free radical in biology, given that O_2 is available, is Fe^{II}, the simple ferrous ion, because together iron and oxygen may generate $OH^{\cdot}$ radicals from oxygen reactions. Ferrous iron must be removed. It has often been suggested that the role of the enzyme caeruloplasmin in blood is for just this purpose: to remove Fe^{II} by converting it to Fe^{III}, which is virtually

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completely bound to transferrin and does not then undergo redox reactions. Another scavenger system is the ascorbate radical which is generated through the ascorbate oxidase reaction of ascorbate. This radical is relatively inert (it is a π free radical), but can react with other, more dangerous, radicals to give disproportionated products. Thus it removes other free radicals, mainly organic in character. The role of ascorbate oxidase could be to maintain a pool of the ascorbate free radical (note the small difference in the ascorbate redox potentials in table 1), which means that the standing potential of ascorbate radical is high. Note also that it is not an aggressive radical kinetically. Both caeroloplasmin and ascorbic acid oxidases are copper proteins, and it would appear again that copper enzymes generally have a special role outside cells, controlling through oxidation a variety of special free radicals and their reactions (Williams 1982).

Free radicals in vesicles and organelles

Higher cells have membrane-enclosed compartments which are not to be confused with the cytoplasm. They are of two kinds: (i) organelles related to primitive prokaryotes; and (ii) vesicles that contain no DNA or RNA. The membranes of both these compartments often have inwardly directed pumps for both proteins and small molecules. It is especially interesting that these pumps remove iron and manganese cations from the cell cytoplasm. Thus the cytoplasm is low in the free radicals Mn^{2+} and Fe^{2+}, presumably to protect eukaryotic DNA from mutation. The DNA of prokaryotes and of mitochondria and chloroplasts can readily allow a higher mutation rate, and the inside of these cells and organelles is relatively unprotected. The pumping of certain metals and certain proteins into these and other organelles generates in them special metallo-enzymes, such as the manganese and iron superoxide dismutases, aconitase, the manganese O_2-generating enzymes (see, for example, Bannister 1982). The other compartments, empty of DNA or RNA, also have Mn^{2+} pumped into them, but they are, in addition, the sites of storage of several haem iron enzymes, for example, catalase in peroxisomes as mentioned above. The protection of the cytoplasm of prokaryotes is therefore from accidental reactions of manganese, iron and copper (all the common free-radical metal ions), but eukaryotes are not so protected. Very different devices are used to remove the metal ions including ion, small chelate-molecule, and protein pumps.

Finally, there is the generation of superoxide and other oxygen radicals in phagosomes, which is part of the general protective activity of leucocytes. Here the reaction is the leakage of reducing equivalents from NADH to flavins and then to oxygen, across a vesicle membrane such that the anion superoxide is trapped inside the vesicle. Its destructive value is then harnessed.

Radicals in membranes

The most obvious parts of a biological system which use free radicals are those concerned with energy capture. Treating the capture of energy from light, there is an initial step, after the transfer of absorbed energy to a reaction centre, which can be written

$$h\nu + P \rightarrow P^* \rightarrow P^{+\cdot} + (A)^{-\cdot}.$$

Here, P* is the excited state of a special reaction-centre complex of two chlorophyll molecules, and $P^{+\cdot}$ is a radical cation at this centre after the escape of an electron to a series of acceptors,

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which become $A^{-\cdot}$ and which may include pheophytins before quinones. The radicals of the quinones have been recognized by their e.p.r. spectra (Rich 1984), and the structure of the reaction complex from certain unicellular organisms is known. Reducing electrons are then given to the $P^{+\cdot}$ state from more remote donors that are usually iron-containing, i.e. free-radical containing proteins; although there is a suggestion that an organic pigment, Z, is involved too. In effect, a free-radical gradient of one-electron redox potential and a gradient of charge is created within the light-harvesting reaction centre and across the containing membrane. Both organic and inorganic centres trapped in membrane proteins are used. These initial reactions are extremely rapid (10^{-12} to 10^{-9} s), but the subsequent transfer of electrons to other acceptors or from donors back to the original centre (all in one-electron steps):

$$\text{donors} \xrightarrow[e]{(2)} P \xrightarrow[e]{(1)} \text{acceptors};$$

and where repeated excitation and steps (1) are followed successively by steps (2), all occur in the millisecond time range. While the first molecules involved in the radical reactions are bound, the molecules of Q in later steps are part of a membrane-contained pool in which they diffuse laterally. It is here that the peculiar protective nature of the medium is so valuable because such radicals as flavin$^{\cdot}$ and quinone$^{\cdot}$ or, for that matter, chlorophyll$^{\cdot}$, are normally readily attacked by oxygen. Inside a protein, especially a metalloprotein, this protection may be managed by steric constraints, but the differing entities in the membrane are at least somewhat exposed. However, in the lipid media it is probable that reaction with oxygen is not so thermodynamically favourable because the product O_2^-, an anion, is not lipid soluble. In fact the radicals of the electron-transfer chain are observed to be relatively stable, reacting only with selected partners within the membrane: a necessity if efficiency of energy capture is to be attained. The very hydrophobic nature of the molecules, such as the Q_{10} quinones, reflects the fact that they are forced to remain in membranes. The switch in the electron-transfer chain from metal to metal (free-radical) electron hops to organic free radicals is also essential to couple the chemistry of electrons to that of protons. Of course it is these dark reactions which lead from the initial charge separation to the generation of proton gradients across and in membranes, because it is the change in redox states of the organic acceptors and donors, unlike those of metal complexes, that lead to cyclic changes in pK_a values on oxidation–reduction:

$$(\text{H-donor}) \rightarrow H^+ + \text{donor}^{\cdot} + e;$$

or, on reduction:

$$H^+ + \text{acceptor} + e \rightarrow (\text{H-acceptor})^{\cdot}.$$

Ultimately these donors and acceptors are involved in two-electron redox reactions and both the oxidation and reduction of the radicals may involve protonation–deprotonation. We must be concerned at this meeting with the coupled acid–base chemistry of radical species (see, for example Rich (1984)) apart from their redox potentials and their disposition in space, because these are seen to be an essential part of energy-capture devices. The dark reactions that occur during light capture also occur in the mitochondrial oxidative reactions: energy capture using O_2–organic molecule reactions. In fact the pathways of redox equivalents, electrons and protons, in mitochondria, chloroplast and bacterial membranes (dark reactions) are remarkably similar. They all have the same functional significance in that they produce initial charge gradients (metals), free-radical gradients, proton gradients, and then ATP or some other chemically trapped energy form (Mitchell 1968; Williams 1961).

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Not all radicals or oxidized molecules in membranes are without hazard and particular risk appears to be associated with organic peroxides which cannot be removed by either catalase or the glutathione-linked systems of the cytoplasm, because these control systems are not lipid soluble. In fact they are removed by vitamin E which is only soluble in lipid phases, and is capable of forming rather innocuous free radicals itself from dangerously reactive ones, just as ascorbate does outside cells and gluthathione may do inside cells.

Radicals in the cytoplasm

A large part of the discussion of free radicals in biology is devoted to the superoxide anion (see other contributions to this symposium). The scavenger enzymes are the Cu–Zn proteins of the eukaryote cytoplasm and the Fe and Mn proteins of prokaryotes, mitochondria and chloroplasts. Elsewhere I have discussed the peculiarities and reasons for this different distribution of enzymes which perform apparently identical tasks but contain different metal ions. The overriding point is the danger from free cytoplasmic Fe^{II} and Mn^{II} to eukaryotes (Williams 1982). I shall not describe superoxide reactions further here. I turn instead to the reactions more often associated with hydrogen peroxide – the intracellular peroxidases – which may well scavenge radicals of a different kind. It must be remembered throughout this section that the production of a lethal mixture of H_2O_2 and O_2^- is an accepted risk, so that its protective value overrides its danger so long as it is produced in the right place. The escaping radicals and peroxides have, however, to be eliminated.

Peroxides are handled by haem enzymes (usually in vesicles), glutathione peroxidase (in the cytoplasm of most cells) and vitamin E (in membranes). As with the multiplicity of superoxide handling proteins there are reasons for the different locations and chemical components of the three classes of reactions (see table 3). The haem enzymes only handle peroxides, mainly H_2O_2,

Table 3. Enzymes destructive to superoxide or peroxides

enzyme	reaction	metal or heavy atom
superoxide dismutase	$O_2^- \rightarrow H_2O_2 + O_2$	Cu(Zn), Fe, Mn
catalase	$H_2O_2 \rightarrow O_2 + H_2O$	Fe(haem)
peroxidase		
phenols	$H_2O_2 + RH \rightarrow ROH + H_2O_2$	Fe(haem)
glutathione	$H_2O_2 + RH \rightarrow ROH + H_2O$ ($2RSH \rightarrow RSSR$)	Se
vitamin E (in membranes)	$RO_2H \rightarrow ROH + H_2O$	none

in free aqueous solution in vesicles, and the reaction follows an enzyme one-electron pathway which would have clear dangers in the cytoplasm. The selenium-containing glutathione peroxidases generally react via an atom-transfer route without giving radicals and can handle all peroxides, even tertiary substituted peroxides, that are soluble in water. They are particularly valuable in the cytoplasm. Neither of these two water-soluble enzymes can control free radicals or peroxides that are organic soluble, so a third, non-enzymic, system is required: vitamin E. The account does not finish here, however, because there is a further protective device in the cytoplasm which links to the glutathione peroxidase. This is the glutathione couple itself, which is the equivalent of a very mild peroxide couple in which oxygen atoms are replaced by sulphur:

$$R_2O_2 \rightarrow 2ROH \quad \text{and} \quad R_2S_2 \rightarrow 2RSH.$$

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The sulphur couple is of much lower redox potential (see table 1). In addition, the sulphur reaction is readily reversible in biology, so the glutathione couple acts as a redox buffer (Flohe 1979). It has the advantage that it can pick up dangerous oxidizing or reducing free radicals while its own radical is not very reactive, so purging the cell cytoplasm. The interesting chemical feature of all these systems is that as we descend Group VI of the Periodic Table from O to S to Se (peroxides, glutathione, glutathione peroxidase), so the chemistry becomes milder (table 1). Organic chemicals open to attack by hydrogen peroxide and radicals are protected by the rapid conversion to the relatively innocuous oxidized derivatives of –SH and –SeH (or to conjugated free radicals such as vitamin E and ascorbate). These two centres are such good scavengers that they, like ascorbate outside cells and vitamin E compounds within membranes, are not dangerous even though their reactive atoms are very exposed. Without free metal ions they do not activate O_2 reduction. The contrast here is with metals (free-radical metals), which can be used to scavenge only when hidden (as for O_2^- or H_2O_2 in catalase and superoxide dismutases), because they cannot resist O_2 reactions and would activate O_2 reduction powerfully if exposed to it.

Radicals in special enzymes

With hindsight it is possible to see that some reactions are extremely difficult to effect in two-electron steps. A typical example is the attack on a carbon atom in a fully saturated chain, $-CH_2-$. Probably the easiest route for this reaction is that of H-atom removal with homolytic radical bond break. Table 4 notes some of the cases which appear to require this pathway and

Table 4. Some radical intermediates in enzymes

enzymic reaction	radicals used
diol rearrangements	Co^{II} B_{12} and adenosine
ribonucleotide reduction	Co^{II} B_{12} and adenosine
	Fe^{III} dimer and tyrosine
haem oxygenases	FeO and porphyrin
cytochrome P_{450}	FeO and $CH^{\cdot}$(substrate)
cytochrome *c* peroxidation	FeO and tryptophan radical or Fe^{III} haem
methanogenesis	Ni^{II}
pyruvate decarboxylation	thiamin and ferredoxin

also lists some of the associated catalysts. The use of metal ions is notable, but it is equally clear that a particular metal ion has been selected for a particular reaction. Cobalt is used in many rearrangements that involve vitamin B_{12}; haem iron is used in many high-potential reactions and polynuclear iron, $Fe_n S_n$, and nickel are used in low-potential reactions. The choice of metal is interesting and must relate to the special nature of these metal ions opposite special organic reaction steps.

Cobalt can be made to yield a very reactive radical with a 3d unpaired electron that is very exposed. This situation is created in low-spin cobalt (II) in a square pyramid five-coordinate geometry. The single unpaired electron is forced into the σ-orbital, d_{z^2} and protrudes from the plane of the complex. This radical, though exposed, is not thermodynamically aggressive and several studies showed it to have a low redox potential. Another interesting feature of the radical is that it is generated in the enzymes, together with a $R-CH_2^{\cdot}$ radical, only when a substrate

is bound (Cockle *et al.* 1972; Finke *et al.* 1984). There is a preferred order of reaction: inert system–substrate binding–activated system (radicals). The unavailability of the cobalt at first prevents attack of oxygen on the cobalt(II). In other words, when the radicals are formed they are highly restricted in their access to reagents within a locked-off enzyme–substrate complex.

One of the reactions which B_{12} enzymes can participate in is ribonucleotide reduction to deoxyribonucleotide. In some organisms this reduction is performed by an alternative (FeOFe)-dependent enzyme. Here the necessary radicals are observed to be present in the enzyme before substrate addition. Apart from the (FeOFe) centre, which contains ten unpaired electrons, there is a tyrosine free radical that is not attacked by oxygen (Sjoberg & Graslund 1978). This remarkable system is not understood. A tyrosine free radical is of high potential and is very reactive, and we must ask how and why it was formed in the enzyme and how it is prevented from reacting indiscriminately.

A rather different scheme can be given for another high redox potential radical: the $Fe^{IV}O$ and the formal $Fe^{V}O$ species that occur in several haem-containing oxygen or hydrogen peroxide using enzymes. The reactions of the enzymes range from phenol or tryptophan oxidation (peroxidases) to hydroxylation of a range of very inert hydrocarbons (P_{450} cytochromes). The initial state of the '$Fe^{V}O$' is really the complex $Fe^{IV}O$ bound to a porphyrin free radical, *or* an $Fe^{IV}O$ complex with an attendant tryptophan free radical, *or* an $Fe^{IV}O$ complex with a second haem unit (free radical), which undergoes only one-electron oxidation (Dolphin & Fenton 1974). These centres undergo either step-by-step one-electron reduction from a substrate *ca.* 1 nm away (peroxidase; Burns *et al.* 1975), or hydrogen-atom uptake and OH loss in an overall two-electron (O-transfer) reaction of the kind $FeO + RH \rightarrow FeOH + R^{\cdot} \rightarrow ROH$. The last reaction requires the RH group to lie immediately on top of the FeO unit. The value of hydroxylation is not just in the destruction of damaging molecules, but in the synthesis of hydroxylated species such as tyrosine and the prostaglandins.

Once again the radicals in these steps, both inorganic and organic, are hidden throughout the reaction cycle. In the case of cytochrome P_{450} the iron atom is not available (low-spin, bound axially to two protein ligands; compare Co^{III} in vitamin B_{12}) until the substrate binds, converting it to an open-sided high-spin iron. The FeO_2 and FeO complexes that are formed subsequently then see the bound substrate, RH, but not any side chain of the protein or any other molecules in solution when they cannot attack the wrong groups. The reaction proceeds in an isolated pocket until the products are generated and it is of great interest that the Fe=O haem unit is activated by a coordinated thiolate (Hill *et al.* 1970). A final example concerns nitrite or sulphite reductase, where the haem iron binds a unit NO_2^- or SO_3^{2-}, which is open to six-electron reduction. The one-electron (radical) steps are assisted by a bound Fe–S centre that, with the haem, makes a two-one electron unit (E. Munck 1984, personal communication).

In table 4 we have included a recent example of a radical pathway in an isolated reaction site which has been uncovered by Kerschev *et al.* (1982). The reaction is that of pyruvate oxidoreduction (figure 1), in which oxidative decarboxylation requires the presence of ferredoxins and the thiamin diphosphate free radical. The ability of enzymes to generate such isolated reaction or carrier pockets is here an elaboration of the haemoglobin story, but is of course common to many acid–base centres such as those of kinase, ATP-synthetase and so on. This parallel stresses the related ways in which enzymes protect reactions, whether they go by radical or non-radical paths.

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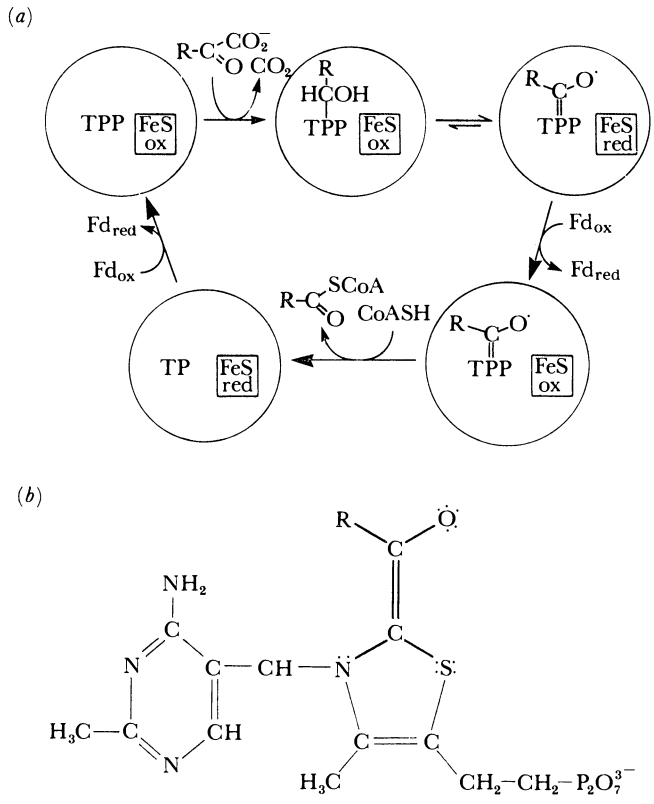

FIGURE 1. (*a*) The proposed reaction cycle of pyruvate oxidoreductase and (*b*) the thiamin radical involved (after Kerscher & Osterhelt 1982).

CONCLUSIONS

Biological systems have many essential or required ways of using free radicals which include:

(i) mechanistically required radical intermediates in different enzymes;

(ii) radicals for free-radical polymerization;

(iii) radicals for energy capture and transduction;

(iv) aggressive radicals for the destruction of invading organisms;

(v) radicals for removing more dangerous radicals.

These reactions carry risks, so all parts of biology need protection from escaping free radicals, as will be discussed in other contributions to this symposium. However, I trust that it is clear that radicals are extremely valuable intermediates in reactions and that their use can be very well controlled. Some free radicals are dangerous, but the whole group of reactions should not be classified by the errant few.

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Discussion

E. C. Baughan (27 *Canonbury Place*, *London N*1). A simple point that is perhaps worth making: ordinary electron-pair reactions differ from free-radical reactions by having a much larger activation energy; for an activation energy of 84 kJ mol^{-1} only one collision in 10^{14} leads to reaction at room temperature. Hence, free radicals in very small concentrations can often dominate the chemical scene. But this relative advantage diminishes with rising temperature, so that a more complex biochemistry becomes possible with organisms that keep themselves warm.

R. J. P. Williams. There is a danger in assuming that biological reactions are like solution reactions. While some molecules are very mobile in cells, some are more or less rigidly held in matrices. We must then look at solid-state reactions as much as at solution reactions. For example, the side chains of proteins such as valine and phenylalanine may have an activation energy for flipping of *ca.* 100 kJ. Thus solvent relaxation could control any reaction rate. The distinctive differences between electron, atom free-radical, or ion transfers seen in solution is not necessarily applicable to the complex matrices of biology. The reason for the tight temperature control of warm-blooded animals could be connected to phase changes of some kind and unrelated to conventional energies of solution kinetics. The question is interesting; the answer unknown.

R. L. Willson (*Department of Biochemistry*, *Brunel University*). I must challenge Professor Williams's statement that damage to proteins is not important but only coincidental. As a generalization I think this could be highly misleading. Clearly many proteins are present in high concentration, and any damage as such may be relatively small and therefore unimportant. Some proteins, however, may be only present in low concentration at some vital period in the life of the cell or tissue, and any damage could have serious repercussions. Polymerase enzymes known to be sensitive to oxidation and vital for the repair DNA damage, chemotactic factors, some of which contain methionine residues sensitive to oxidation, or protease inhibitors which in some cases are also sensitive to oxidation and which are thought to be involved in tumour promotion, are just some examples.

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R. J. P. Williams. I accept the general statement that damage to any essential molecule that is present as a single or as very few copies in a cell is likely to be serious. DNA is *the* example. It is large and unique and most of its sequence must be faithfully retained. Radiation damage to DNA is undoubtedly very serious. Damage to proteins by radiation is far less likely to be serious for several reasons: most of such proteins exist as multiple copies; individually they are small molecules; a reasonable fraction of their amino acids can be damaged without affecting activity; and finally they can be reproduced. A quantitative examination of the probability of causing any damage to polymerase enzymes in a cell based on target size must give a value less than 0.1% of the likelihood of causing damage to DNA. At the same time there will be some thousand-fold greater coincidental damage to proteins, taking all the proteins of the cell. With this proviso my statement stands and I remain unconvinced of the value of the study of radiation damage to proteins.

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