Free-radical pathology and medicine

A review

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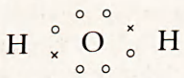
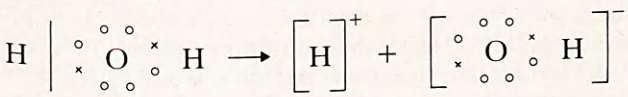
Retrospect

Free radicals are a chemical species with an unpaired electron in their structure. For many years this meant that they were forbidden to exist. The theoretical foundations of modern chemistry were laid toward the end of the 19th century; and the obligatory pairing of electrons, closely related to the doctrines of valency and molecular weight, was part of these foundations. The explanation is very complex or very simple. Though electrons are best envisaged as negative charges orbiting around positively charged nuclei, each electron also has a 'magnetic moment', ie it behaves like one pole of a magnet. One of the enduring wonders of school physics is that, however many times a magnetised rod is divided between its north and south poles, each fragment will instantly re-form the missing pole. Similarly, stable molecules and ions in solution will always maintain—or try to maintain—a paired electron complement.

True to this rule, an ordinary chemical bond, conventionally written as a dash, is a two-electron structure to which each side contributes one electron. Water can be represented as

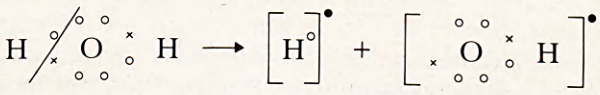


where each circle is an electron belonging to the oxygen atom and the crosses are electrons contributed by the hydrogen atoms. Even when, in the course of a chemical reaction, such a bond splits, electron pairing is preserved. The ionisation of water, for example, generates two fragments: the hydroxy fragment carries off both bonding electrons and the hydrogen fragment is left with none:



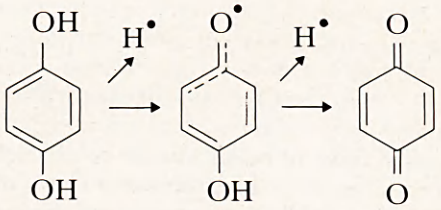
(The rules of roulette do not apply; nought counts as 'paired'.) In chemical parlance this is heterolytic fission.

The law of electron pairing had hardly been enunciated when apparent exceptions began to be noticed. In a celebrated review just over 50 years ago Hey and Waters interpreted several such anomalies in terms not of heterolytic but of equal or homolytic fission [1]. If this happened to water the products would be two fragments, each with an unpaired electron—a hydrogen atom and a hydroxy free radical:



(Unpaired electrons are conventionally symbolised by a dot.)

About the same time Michaelis, eponymous hero of enzyme kinetics, suggested that in some oxidation–reduction reactions the transfer of the electron pair (in the form of two hydrogen atoms) from reductant to oxidant occurred in two stages. The intermediate stage had to be a free radical [2]:



It does not detract from the boldness of this assertion that these two-stage transfers were not free-radical reactions in the currently accepted sense: they involved the temporary separation of an electron pair but not the interaction of a free radical with a previously stable molecule. The animals, in other words, still had to enter Noah's Ark in pairs but they could now proceed in a single file. More relevant to current free-radical concepts was the work of Farmer and his group during World War II (sadly overshadowed by the contemporary development of penicillin and the atomic bomb). In a series of papers they unravelled the age-old mystery of rancidification—the massive, irreversible and seemingly unpredictable breakdown of unsaturated fats and oils [3]. The self-catalysing chain reaction they described still represents a key to free-radical behaviour.

Let it be assumed that at irregular intervals in a lipid suspension a single electron (in the form of a

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hydrogen atom) is abstracted from the hydrocarbon chain of a lipid molecule (L_1). This, by definition, creates a lipid free radical ($L_1\cdot$). In the absence of oxygen the event would be of little consequence. Free radicals attract each other, and in the fullness of time two lipid free radicals would collide, interact and, by gaining or losing a single electron, mutually restore their electron pairing. But for complex reasons oxygen molecules, ie two linked oxygen atoms or O_2, behave in some respects not like molecules but like a pair of free radicals stuck together. Such 'biradicals' are almost as strongly attracted to free radicals as are other free radicals. In a lipid suspension at equilibrium with air, oxygen molecules are of course everywhere. One such molecule will instantly interact with the lipid free radical, generating a new free-radical species—the lipid-peroxy free radical:

$$L_1^{\bullet} + O_2 \longrightarrow L_1{-}OO^{\bullet}$$

Lipid-peroxy free radicals are far more aggressive in seeking a single electron than ordinary lipid free radicals; instead of waiting for another lipid free radical to come along, they will tear an electron from a previously stable lipid molecule. With its paired electron complement thus restored, the lipid-peroxy free radical becomes a lipid peroxide molecule, but a second lipid free radical has been generated. The second lipid free radical will at once interact with a second oxygen molecule to generate a second lipid-peroxy free radical, and the second lipid-peroxy free radical will disrupt a third lipid molecule. So the chain continues:

$$L_1{-}OO^{\bullet} + L_2 \longrightarrow L_1{-}OOH + L_2^{\bullet}$$
$$L_2^{\bullet} + O_2 \longrightarrow L_2{-}OO^{\bullet} \quad \text{etc}$$

Lipid peroxides, though not of course free radicals, are themselves unstable; spontaneously (often involving other free-radical reactions) or enzymically they break down to an unstable constellation of smaller fragments, the source of the characteristic smell and taste of rancid fat.

The understanding of this autocatalytic process revolutionised the food industry. Within a few years it also transformed everyday life. All plastics and polymers—fabrics, glues, paints, explosives, the list is almost endless—are the products of self-catalysing free-radical chain reactions, and their control became a prime objective of industrial chemists. Indeed, almost the only branch of chemistry that seemed free-radical-proof was biochemistry. Szent-Györgyi speculated seductively about foot-loose electrons in biological material [4], and a few pioneers began to explore their possible pathological significance [5, 6], but as recently as the early 1960s a speculative article in *The Lancet* on 'biological rancidification' provoked an eminent and very cross correspondent to state that 'free-radical reactions are inherently incompatible with organised life'.

These not-so-remote attitudes are worth recalling not for their tepid anecdotal interest but for their continued relevance. Attitudes in medicine often move from one extreme to the other and free radicals are now moving centre-stage. Ironically perhaps the discovery of superoxide dismutase (SOD) was a great populariser [7]. Although the enzyme is important, its impact probably owes more to the kind of reasoning scientists usually deplore. Since SOD uses the superoxide free radical as its substrate, the superoxide free radical must also, at least potentially, exist [8], and if one free radical exists, why should not others? It is therefore useful to remember that many of the objections to their existence are as valid today as they were 25 years ago. Free radicals *are* an 'improbable' biological species; their detection *is* fraught with difficulties; and, perhaps most important, their biological 'role' *is* different from that of any other chemical breed.

Characteristics

Improbability

In the lipid autoxidation sequence outlined above, free radicals seem to tumble over each other, but the scheme is introduced with the words 'let it be assumed'. Such exhortations should never be allowed to pass unchallenged. This particular one glosses over the fact that, while a single free radical can trigger off a fast (sometimes explosively fast) free-radical chain reaction, the mechanism that brings forth the initiating species usually remains obscure. In a lump of butter there are probably three necessary ingredients. First, polyunsaturated lipids have a number of double bonds in their hydrocarbon chain, and double bonds encourage the momentary drifting apart of electrons. Second, such material always contains traces of transition metals (eg Fe, Cu) which gain or lose a single electron in transit between valency states. Third, oxygen can convert a temporary estrangement between electrons into a permanent separation. Even though these ingredients are all present in organised biological systems, the essence of organisation is that they are kept strictly apart. Moreover, especially vulnerable structures such as lipid membranes are lavishly provided with protective mechanisms [9]. Underlying these difficulties is the problem of the energy source. The preference of electrons to remain in pairs means that a correspondingly strong wrench, ie a large single quantum of energy, is needed to separate them. This can be provided from the outside by ionising or ultraviolet irradiation; it is how radiation works. But few if any of the enzymic reactions which make up the interlocking cycles of normal intermediate metabolism cater for such quanta. (Massed minutiae sometimes obscure the fact that the central purpose of intermediate metabolism is the parcelling up of food energy into minute quanta.) How then are biological free-radical reactions initiated in a normally functioning cell? The answer—with a few important but isolated exceptions—may be that they are not.

That free radicals can damage organised living tissue was one of the opening salvos of free-radical biochemistry, but it is still not generally recognised that the relationship is reciprocal. The essential ingredient which is, by definition, missing from a normally functioning cell is structural damage. It may not matter much what the damaging agent is, whether it is mechanical, chemical, immunological, microbiological or simple wear and tear, but some degree of structural disruption is probably essential. There are two reasons for this. First decompartmentalisation allows the components of free-radical generation actually to interact. Second, in terms of energy structural disruption is always, to a greater or lesser extent, a reversal of structural construction. Whatever individual steps lead to the construction of a biological unit, the overall balance is always highly energy-consuming. Much of this energy remains latent in the finished product. When the product is damaged or destroyed some of it is released. The event may provide the explosive force necessary to tear an electron pair apart.

Detection

All free radicals of biological significance have a lifespan of the order of milliseconds or less, and even in a lump of fat actually going rancid the concentration of unpaired electrons at any moment is far too small to be detectable by conventional chemical methods. The only way of catching the species *in flagrante* is by electron-spin resonance (ESR) spectroscopy. The tool required is a powerful electromagnet whose magnetic field is deflected by the unbalanced magnetic pole of unpaired electrons. It is, as medical research grants go, expensive, and considerable expertise is required to interpret signals from different free radicals. Also, biological sample preparation is not easy since the free radicals present at any moment have to be trapped by instant freezing. (Free radicals can also be trapped chemically over an extended period by letting them form relatively stable free-radical adducts with certain groups of chemicals [10]. Unfortunately the chemical traps available at present are highly toxic.) All these difficulties are gradually being overcome: ESR spectroscopy has already been successfully applied to the study of cancer of the human uterus [11]. But on a wide front biological free-radical research will have to rely for many years yet on indirect evidence. There are several possible approaches.

First, although free radicals as such may be undetectable, some products of free-radical mediated reactions are relatively stable and can therefore serve as 'markers'. Almost 50 years ago a human brain removed at necropsy was inadvertently (one presumes) left lying on the laboratory bench. It developed a 'storage' product which gave a pink colour when heated with thiobarbituric acid (TBA) [12]. The product was subsequently identified as malonyldialdehyde (MDA) and later as a mixture of lipid peroxidation products all of which break down to MDA in the assay [13]. The compound or mixture of compounds is still the most widely used free-radical marker, and the improved assay gives a fairly accurate and reproducible if essentially comparative estimate of free-radical mediated lipid peroxidation [14, 15]. Under appropriately controlled conditions the measurement of 'diene conjugation', a characteristic shift in the double-bond sequence of free-radical damaged lipids, can be both more sensitive and more specific, but dietary and other extraneous influences have to be taken into account [16]. Free-radical damaged lipids also release small, volatile hydrocarbon fragments such as ethane and pentane, and these can be measured in the gas phase of an experiment or in expired air [17]. Quite subtle changes in proteins exposed to free-radical activity are detectable under certain conditions by monitoring changes in their fluorescence characteristics [18].

Second, on the assumption that cells and tissues *in vivo* live under the constant threat of uncontrolled free-radical activity and therefore need elaborate protection, the effectiveness of one or several protective mechanisms can be measured. The results are often interesting but ambiguous. The decreased concentration of a 'protective' enzyme (eg SOD) can be interpreted as suggesting increased free-radical activity but, since enzyme concentrations tend to be partly governed by the amount of substrate provided by the system, it may also suggest the exact reverse. One hopes at least for some consistency.

The need for constant and effective protection also underlies a variety of assays in which cells or tissues are exposed to free-radical generating stress and their response is measured. Red blood cells, for example, in a wide range of haemolytic diseases generate significantly more TBA-reactive material than normal red cells when exposed to high concentrations of hydrogen (or organic) peroxide [19]. Conversely, plasma or other biological fluids can be added to a test system undergoing free-radical autoxidation and their antioxidant potency measured [20, 21].

Other approaches can be useful in limited, mostly experimental fields. The oxygen electrode can record the dramatic burst of oxygen uptake (misnamed 'respiratory' burst) in polymorphonuclear and other phagocytic cells. It reflects the massive generation of oxygen-derived free radicals, an essential step in the destruction of engulfed microorganisms [22]. The mechanism is defective in chronic granulomatous disease so that the engulfed organisms survive [23]. Electron microscopy can beautifully display the characteristic blebbing of cell membranes damaged by free radicals [24]. Chemiluminescence is a useful if still somewhat non-specific tool [25]. More indirectly, changes in the concentration of protein thiols or in the composition and relative concentration of polyunsaturated and saturated lipids can give a hint of free-radical activity [26, 27]. To all these methods—and the above list is by no means exhaustive—the general rule of indirect evidence applies. The data can be valuable provided one knows (more or less) what one is measuring. The reverse is equally true.

Role

At the risk of generalising in a field where general statements tend to return like boomerangs and flatten the generaliser, there may yet be some merit in trying to contrast free-radical and classical, ie enzymic, biochemistry. Although enzymes can and do act in free solutions, their special qualities are best displayed when they are part of a functioning organised biological structure. In such structures their action is continuous and repetitive but also flexible, a complex hierarchy of regulators ensuring that their rate of activity is finely attuned both to external pressures and to internal demand. This enables them to maintain the structural and functional constancy of the unit of which they are part. Insofar as one can ascribe to them a single overriding objective, it is to process the energy in food into energy for living cells to use (either immediately or after a period of storage) to accomplish their varied biological tasks. The simplest way of characterising free-radical mediated processes is to say that none of these generalisations applies to them.

In contrast to the essentially homeostatic action of enzymes, free-radical reactions tend to change a system rather than preserve it, and the change often means destruction. They are trigger mechanisms (whether they trigger off a single shot or a volley of shots) and the effect is usually unrepeatable and irreversible. In terms of energy economy they are no more useful than an explosion in a fuel tank. Although their activity can be influenced by 'promoting' and 'inhibitory' agents—eg antioxidants, vitamins, some specialised proteins, metal chelators—they are not susceptible to continuous fine monitoring and regulation. Such attributes may conjure up a somewhat negative image, and at the cellular level there is indeed some justification for regarding them as agents and products of doom. They are so regarded by many biochemists, but in the context of complex organisms —and that means human pathology and medicine— the opposite is true.

The survival of a complex organism depends most critically perhaps on the continuous destruction of its constituent units. The reason is not just the survival value of processes like phagocytosis and platelet clumping or the obvious need to eliminate damaged, inefficient and potentially harmful cells. In general terms destruction is a precondition of normal turnover. Theoretically, it has to be said, a balanced turnover could be achieved from the production end just as easily as from the destruction end; the rate of production of new cells, for example, could set the rate of destruction of old ones. It just happens that this is not how nature operates. In the human body, without any known exception, it is always the rate of destruction that determines the rate of production. Without an efficient self-destruct mechanism built into every cell and without a feedback system activated by this mechanism there would therefore be no cell turnover, and without cell turnover there would be no survival. (Since this is also true of subcellular particles the principle also applies to tissues with an apparently stable cell population.) There is much circumstantial evidence, though no proof, that the self-destruct mechanism, one of the most important and least understood in biology, is triggered by the generation of a free radical or a sequence of free radicals.

Free radicals and disease

The perceived role of free-radical reactions in disease has changed beyond recognition over the past few years and there is now hardly a pathological process where free radicals are not being sought and usually found. No reviewer can hope to do more than pick out a few examples.

Reperfusion injury

Trendy subjects are not necessarily without lasting importance. Since oxygen is a powerful promoter of free-radical generation, tissue ischaemia and the consequent anoxia is not, on the face of it, a promising target for free-radical research. The fallacy of this argument was first demonstrated by McCord [28]. He showed that, while tissue anoxia is indeed unfavourable to free-radical generation, following a period of ischaemia (and perhaps surrounding an area of ischaemia) there is often a tremendous outburst of free-radical activity. It is possible, as McCord originally suggested, that under ischaemic conditions certain enzymes which do not normally generate free radicals change their character and become potential free-radical generators (xanthine dehydrogenase, in particular, may become a xanthine oxidase) and that the change becomes manifest when oxygen is readmitted. But there may be other and perhaps more important factors which contribute to the free-radical explosion, eg the appearance and activation of phagocytic cells, part of the inflammatory response to ischaemia, or, more generally, the structural damage and destruction suffered by ischaemic tissue. Whatever the mechanism, the concept may have wide clinical implications. Post-ischaemic and perhaps peri-ischaemic changes have long been recognised as significant contributors to the morbidity and mortality of acute ischaemic episodes— whether in the heart muscle, the liver, the bowel, the brain or the spinal cord—and the prevention or mitigation of these changes by introducing free-radical scavengers is clearly a possibility which needs to be explored [29–33].

Cancer

Cancer is a two-faced problem. A wide range and variety of 'causes' are capable of initiating and promoting it; but, more elusively, there are also powerful mechanisms, some almost certainly built into every cell, which self-destruct units that have undergone potentially cancerous change. Failure of the latter mechanisms may be at the root of many forms of *clinical* cancer [34]. Numerous and powerful links have been

established over many years between free radicals and cancer initiation and promotion, ranging in origin from the electronic structure of aromatic compounds to the practicalities of radiotherapy and cancer chemotherapy [35, 36]. Possible links between free-radical activity and the second face of cancer are more recent. Much evidence now suggests that cancer cells are more resistant to lipid peroxidation (better 'protected', to use an increasingly meaningless terminology) than normal cells [37–40], and the possibility has to be envisaged that in some sites and tissues progress from cellular to clinical cancer may depend on the failure of this free-radical mediated self-destruct process [40].

Atheroma

Atheromatous plaques are loaded with lipid peroxides and lipid peroxidation products, a tantalising fact known for many years [41]. A more important recent observation has been the free-radical mediated peroxidation of lipids in circulating LDL and the selective scavenging of such complexes by macrophages [42, 43]. The factors that determine or influence this peroxidation [44, 45] need to be identified.

Ageing

The basic question about ageing, rarely formulated as clearly as it should be, is: does it exist? In other words, is ageing the sum total of a wide variety of age-related diseases (from baldness to cancer), or are most of the age-related diseases manifestations of a distinct and potentially identifiable biological process? Insofar as the latter is nearer the truth, free radicals could play a significant part in it. The symptomless accumulation of age pigments in many organs is probably the most constant biochemical marker of chronological age, and lipid peroxidation products and free-radical damaged proteins make up the bulk of this material [46]. Such pigments characteristically accumulate in a number of rare diseases associated with premature ageing, and some variants are susceptible to treatment or at least control by antioxidant therapy [47].

Inflammatory disease

The acute inflammatory response illustrates both the importance and the elusiveness of free-radical reactions. There can be little doubt that they trigger many of the characteristic changes that follow tissue damage and destruction, but the link between them and the emergence of a host of chemical regulators and messengers—including prostaglandins, leukotrienes and other still unidentified 'factors'—is still far from clear [27]. Free-radical reactions may have a no less critical role in chronic inflammatory states. For many years free-radical biochemistry has been dominated by lipids, a legacy perhaps of organic and industrial chemists preoccupied with the autoxidation of fats and oils. Only in the past few years has the study of free-radical damage to proteins taken wings [48, 49]. Considerable evidence now suggests that free-radical attack may subtly but significantly alter the immunological properties of immunoglobulins and that increased free-radical activity may be the underlying cause of the chronicity of rheumatoid and other sustained inflammatory states [49].

Toxic states

Some of the pioneering studies of free radicals as a cause of tissue damage were conducted on animals exposed to carbon tetrachloride [50, 51], and carbon tetrachloride poisoning remains an important experimental model [52]. Outside the laboratory ethanol is a more popular organic solvent, and marked increase in free-radical activity has been reported in chronic intoxication [53, 54]. The link may be especially strong with alcoholic myopathy [55]. The usually irreversible and often fatal effects of paraquat poisoning are almost certainly caused by free radicals [56, 57]. Free radicals may also play a part in endotoxic shock [58], and preliminary evidence suggests a link between increased free-radical activity and preeclamptic toxaemia [59]. The detoxification by normal organs of potentially harmful chemical agents (often lumped together as 'xenobiotics') is a half-way house between normal and abnormal biochemistry and one of the areas where enzymic and free-radical processes closely overlap. Many detoxification reactions involve molecular oxygen, and oxygen-derived free radicals may be important intermediates or side-products [60].

Other diseases

At this point one cannot but succumb to pathological name-dropping. The development of both types of diabetes and of some important diabetic complications have been persuasively linked to free radicals [61, 62]. The action of the malaria parasite in red cells and the action of some antimalarial drugs involve free radicals [63]. Some aspects of chronic pancreatic disease have been brilliantly interpreted in free-radical terms [64]. There can be little doubt that most of the toxic manifestations of iron overload depend on the catalytic effect of 'free' iron on free-radical generation [65]. Infertile or subfertile human spermatozoa have a markedly increased oxygen free-radical response to stimulation with NADPH [66]. Free-radical activity may prove to be an important variable in determining the survival of organs for transplantation [67]. Such a list could easily be continued even without recourse to that universal whipping boy of medical ignorance, cigarette smoke (an arsenal of free radicals). But what does it all add up to?

Prospect

There can be few biochemistry departments unadorned by one of those immensely complicated metabolic charts which purport to represent and sum-

marise the workings of the ideal cell. Even on recent products one looks in vain for free radicals. Does that mean that the charts are wrong or that free radicals do not exist? The answer is neither; the real figment is the 'ideal cell'. In all organisms above the simplest, cells are continuously being damaged or destroyed by a wide range of noxious agents. When they are not being damaged or destroyed they are growing old, and when they are neither being damaged or destroyed nor growing old they are reproducing themselves. More and more evidence suggests that it is these changes—all departures from the 'ideal' of chart-makers—which involve, depend on or are governed by free radicals; and they are all potential initiators of disease. This is why free-radical research is becoming both easier and more difficult. It is becoming easier because in investigating almost any aspect of human pathology one need no longer fear a complete blank. But it is also becoming more difficult because to be able to conclude that in any abnormal process free radicals are 'involved'—a conclusion that provoked surprise and even incredulity 10 years ago—is no longer enough. What free radicals are involved; and how; and can their involvement be influenced? To answer such questions much of the old, indirect and essentially non-specific methodology needs to be up-dated. At the clinical level, if present and future methodology is to be properly directed, the scope of free-radical pathology will have to be better understood. It is a challenging prospect but also an exciting one.

References

1. Hey, D.H. and Waters, W.A. (1937) Some organic reactions involving the occurrence of free radicals in solution. *Chemical Reviews*, **21**, 169–80.
2. Chance, B. (1961) Free radicals and enzyme substrate compounds. In *Free radicals in biological systems* (ed. N.S. Blois) pp 1–14. London: Academic Press.
3. Walling, C. (1957) In *Free radicals in solution*, pp 1–43. New York: Wiley.
4. Szent-Györgyi, A. (1960) *Introduction to submolecular biology*. London, New York: Academic Press.
5. Wills, E.D. (1966) Mechanism of lipid peroxidation in tissues. *Biochemical Journal*, **99**, 667–76.
6. Slater, T.F. (1972) *Free radicals in tissue injury*. London: Pion.
7. McCord, J.M. and Fridovich, I. (1968) The reduction of cytochrome c by milk xanthine oxidase. *Journal of Biological Chemistry*, **243**, 5753–61.
8. Dormandy, T.L. (1979) Free radical reactions in biological systems. *Annals of the Royal College of Surgeons of England*, **60**, 188–94.
9. Dormandy, T.L. (1978) Free-radical oxidation and antioxidants. *Lancet*, **i**, 647–51.
10. Janzen, E.G. (1980) A critical review of spin trapping in biological systems. In *Free radicals in biology* (ed. W.A. Pryor) pp 115–54. New York: Academic Press.
11. Benedetto, C., Bocci, A., Dianzani, M.U. *et al.* (1981) Electron spin resonance studies on normal human uterus and cervix and on benign and malignant uterine tumours. *Cancer Research*, **41**, 2936–42.
12. Kohn, H.I. and Liversedge, M. (1944) A new aerobic metabolite whose production by brain is inhibited by apomorphine, emetine and menadione. *Journal of Pharmacology*, **82**, 92–9.
13. Gutteridge, J.M.C., Stocks, J. and Dormandy, T.L. (1974) Thiobarbituric acid reacting substances derived from autoxidising linoleic and linolenic acid. *Analytica Chimica Acta*, **70**, 107–11.
14. Yagi, K. (1976) A simple fluorimetric assay for lipid peroxide in blood plasma. *Biochemical Medicine*, **15**, 212–6.
15. Pryor, W.A. (1987) Detection of lipid peroxidation products. *Journal of Free Radical Biology and Medicine*, **3**, 317–64.
16. Dormandy, T.L. and Wickens, D.G. (1987) The experimental and clinical pathology of diene conjugation. *Chemistry and Physics of Lipids*, **45**, 353–64.
17. Gee, D.L. and Tappel, A.L. (1981) Production of volatile hydrocarbons by isolated hepatocytes. *Toxicology and Applied Pharmacology*, **60**, 112–20.
18. Wickens, D.G. and Dormandy, T.L. (1984) The effect of free radical activity on human gamma globulins. *Agents and Actions*, **15**, 47–8.
19. Stocks, J. and Dormandy, T.L. (1971) The autoxidation of human red cell lipids induced by hydrogen peroxide. *British Journal of Haematology*, **20**, 95–111.
20. Stocks, J., Gutteridge, J.M.C., Sharp, R.J. and Dormandy, T.L. (1974) The inhibition of lipid autoxidation by human serum and its relation to serum proteins and tocopherol. *Clinical Science and Molecular Medicine*, **47**, 223–33.
21. Cranfield, L.M., Gollan, J.L., White, A.G. and Dormandy, T.L. (1979) Serum antioxidant activity in normal and abnormal subjects. *Annals of Clinical Biochemistry*, **16**, 299–306.
22. Babior, M.B. (1978) Oxygen-dependent microbial killing. *New England Journal of Medicine*, **298**, 659–80.
23. Segal, A.W. and Peters, T.J. (1976) Chronic granulomatous disease. *Lancet*, **i**, 1363–70.
24. Smith, M.T., Thor, H., Jewell, S.A. *et al.* (1984) Free radical induced changes in the surface morphology of isolated hepatocytes. In *Free radicals in molecular biology* (ed. D.A. Armstrong, R.S. Sohal, R.G. Cutler and T.F. Slater) pp 113–20. New York: Raven.
25. Boveris, A., Cadenas, E., Reiter, R. *et al.* (1980) Organ chemiluminescence: non-invasive assay for organic radical reactions. *Proceedings of the National Academy of Medical Sciences of the USA*, **7**, 347–51.
26. Slater, T.F., Bajardi, F., Benedetto, C. *et al.* (1985) Protein thiols in normal and neoplastic human uterine cervix. *FEBS Letters*, **187**, 267–71.
27. Halliwell, B. and Gutteridge, J.M.C. (1985) *Free radicals in biology and medicine*. Oxford: Clarendon Press.
28. McCord, J.M. (1985) Oxygen-derived free radicals in post-ischaemic tissue injury. *New England Journal of Medicine*, **312**, 159–63.
29. Davies, M.J., Garlick, P.B., Slater, T.F. and Hearse, D.J. (1988) Free-radical production during myocardial reperfusion injury using electron-spin-resonance spin trapping. In *Free radicals: chemistry, pathology and medicine* (ed. C. Rice-Evans and T.L. Dormandy) pp 303–21. London: Richelieu.
30. Granger, D.N., Hollwarth, M.E. and Parks, D.A. (1986) Ischemia-reperfusion injury: role of oxygen-derived free radicals. *Acta Physiologica Scandinavica*, **548** Suppl., 47–63.
31. Nilsson, U.A., Olsson, L.I., Thor, H. *et al.* (1989) Determination of oxygen free radicals during reperfusion of intestinal cells *in vitro*. *Free Radical Biology and Medicine*, **6**, 251–9.
32. Parks, D.A., Bulkley, G.B., Granger, G.B. *et al.* (1982) Ischaemic injury in the cat small intestine: role of superoxide radicals. *Gastroenterology*, **82**, 9–15.
33. Braughler, J.M. and Hall, E.D. (1989) Central nervous system trauma and stroke. *Free Radical Biology and Medicine*, **6**, 289–313.
34. Iwasa, K., Ikata, T. and Fukuzava, K. (1989) Protective effect of vitamin E on spinal cord injury by compression and concurrent lipid peroxidation. *Free Radical Biology and Medicine*, **6**, 599–606.
35. McBrien, D.C.H. and Slater, T.F. (ed.) (1982) *Free radicals, lipid peroxidation and cancer*. London: Academic Press.
36. Connors, T.A. (1989) The involvement of free radicals and lipid peroxidation in carcinogenesis. In *Eicosanoids, lipid peroxidation and cancer* (ed. S.N. Nigam, D.C.H. McBrien and T.F. Slater) pp 143–51. Berlin: Springer-Verlag.
37. Thiele, E.H. and Huff, J.W. (1960) Lipid peroxide production and inhibition by tumour mitochondria. *Archives of Biochemistry and Biophysics*, **88**, 208–11.
38. Utsumi, K., Yamamoto, G. and Inaba, K. (1965) Failure of Fe(II) induced lipid peroxidation and swelling in the mitochondria isolated from ascites tumour cells. *Biochimica et Biophysica Acta*, **105**, 368–71.

39. Cheeseman, K.H., Collins, M., Proudfoot, K. *et al.* (1986) Studies on lipid peroxidation in normal and tumour tissues. *Biochemical Journal*, **235**, 507–14.
40. Dormandy, T.L. (1988) In praise of peroxidation. *Lancet*, **ii**, 1126–9.
41. Glavind, J., Hartmann, S., Clemmensen, J. *et al.* (1952) Studies on the role of lipoperoxides in human pathology; the presence of peroxidised lipids in the atherosclerotic aorta. *Acta Pathologica et Microbiologica Scandinavica*, **30**, 1–6.
42. Szczeklik, A., Gryglewski, R.J., Domagala, B. *et al.* (1981) *Prostaglandins*, **22**, 785–95.
43. Esterbauer, H., Quekenberger, O. and Jurgens, G. (1988) Effect of peroxidative conditions on human low-density lipoproteinsIn *Eicosanoids, lipid peroxidation and cancer* (ed. S.K. Nigam, D.C.H. McBrien and T.F. Slater) pp 203–13. Berlin: Springer-Verlag.
44. Belch, J.J.F., Chopra, M., Hutchinson, S. *et al.* (1989) Free radical pathology in chronic arterial disease. *Free Radical Biology and Medicine*, **6**, 375–8.
45. Stringer, M.D., Görög, P.G., Freeman, A. and Kakkar, V.V. (1989) Lipid peroxides and atherosclerosis. *British Medical Journal*, **298**, 281–4.
46. Siakatos, A.N. and Munkres, K.D. (1982) Recent developments in the isolation and properties of autofluorescent lipopigments. In *Ceroid lipofuscinosis (Batten's disease)* (ed. D. Armstrong, N. Koppang and J.A. Rider) pp 165–87. Amsterdam: Elsevier Biomedical Press.
47. Westermarck, T., Santavouri, P., Marklund, S. *et al.* (1982) Studies on the effect of selenium administration to neuronal lipofuscinosis patients. In *Ceroid lipofuscinosis (Batten's disease)* (ed. D. Armstrong, N. Koppang and J.A. Rider) pp 392–407. Amsterdam: Elsevier Biomedical Press.
48. Wickens, D. G., Norden, A.G. and Lunec, J. (1983) Fluorescence changes in human gamma globulin induced by free-radical activity. *Biochimica et Biophysica Acta*, **742**, 607–16.
49. Griffith, H.R., Unsworth, D., Blake, D. and Lunec, J. (1988) Oxidation of aminoacids within serum proteins. In *Free radicals: chemistry, pathology and medicine* (ed. C. Rice-Evans and T.L. Dormandy) pp 439–54. London: Richelieu.
50. Slater, T.F. (1966) Necrogenic action of carbon tetrachloride in the rat: a speculative mechanism based on activation. *Nature*, **209**, 36–40.
51. Recknagel, R.O. and Ghoshal, A.K. (1966) Lipoperoxidation as a vector in carbon tetrachloride hepatoxicity. *Laboratory Investigations*, **15**, 132–46.
52. Poli, G., Cheeseman, K.H., Dianzani, M.U. and Slater, T.F. (ed.) (1955) *Free radicals in liver injury*. Oxford: IRL Press.
53. Fink, R., Marjot, D.H., Cawood, P. *et al.* (1985) Increased free-radical activity in alcoholics. *Lancet*, **ii**, 291–4.
54. Peters, T.J., O'Connell, M.J., Venkatesan, S. and Ward, R.J. (1986) Evidence for free-radical mediated damage in experimental liver disease. In *Free radicals: cell damage and disease* (ed. C. Rice-Evans) pp 99–110. London: Richelieu.
55. Ward, R.J., Duane, P.D. and Peters, T.J. (1988) Nutritional selenium and alpha-tocopherol status of alcohol abusers with and without chronic skeletal myopathy. In *Alcohol toxicity and free radical mechanisms* (ed. R. Nordman, C. Ribière and H. Rouache) pp 93–100. Oxford: Pergamon.
56. Shu, H., Talcott, R.E. and Rice, S.A. (1979) Lipid peroxidation and paraquat toxicity. *Biochemical Pharmacology*, **28**, 327–31.
57. Richmond, R. and Halliwell, B. (1982) The formation of hydroxyl radicals from the paraquat radical cation, demonstrated by a highly specific gas chromatographic technique. *Journal of Inorganic Biochemistry*, **17**, 95–9.
58. Stark, J.M., Jackson, S.K. and Parton, J. (1988) Increases in antioxidants after endotoxin in mice. In *Free radicals: chemistry, pathology and medicine* (ed. C. Rice-Evans and T.L. Dormandy) pp 187–210. London: Richelieu.
59. Wickens, D.G., Wilkins, M.H., Lunec, J. *et al.* (1981) Free radical oxidation (peroxidation) products in plasma in normal and abnormal pregnancy. *Annals of Clinical Biochemistry*, **18**, 158–62
60. Mason, P.R. (1982) Free radical intermediates in the metabolism of toxic chemicals. In *Free radicals in biology*, Vol. 2 (ed. W.A. Pryor) pp 159–78. New York: Academic Press.
61. Oberley, L.W. (1988) Free radicals and diabetes. *Free Radical Biology and Medicine*, **5**, 113–24.
62. Collier, A., Jackson, M., Dawkes, R.M. *et al.* (1988) Reduced free radical activity detected by decreased diene conjugates in insulin-dependent diabetic patients. *Diabetic Medicine*, **5**, 747–9.
63. Clark, I.A., Chaudhri, G. and Cowden, W.B. (1989) Some roles of free radicals in malaria. *Free Radical Biology and Medicine*, **6**, 315–22.
64. Braganza, J. (1988) Free radicals and pancreatitis. In *Free radicals: chemistry, pathology, medicine* (ed. C. Rice-Evans and T.L. Dormandy) pp 357–82. London: Richelieu.
65. Gutteridge, J.M.C. (1988) Lipid peroxidation: some problems and concepts. I. Oxygen radicals and tissue injury (ed. B. Halliwell). Bethesda: Federation of American Societies for Experimental Biology.
66. Aitken, R.J. and Clakson, J.S. (1988) The generation of reactive oxygen species by human spermatozoa. In *Free radicals: chemistry, pathology, medicine* (ed. C. Rice-Evans and T.L. Dormandy) pp 187–210. London: Richelieu.
67. Fuller, B.J. and Green, C.J. (1986) Oxidative stress in organs stored at low temperature for transplantation. In *Free radicals: cell damage and disease* (ed. C. Rice-Evans) pp 223–40. London: Richelieu.