Free Radicals in Biology

VOLUME V

Edited by
William A. Pryor

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General Preface

This multivolume treatise had its genesis in April, 1970, when a number of chemists and biologists interested in free radical biology met in Atlantic City at the President's Symposium of the American Society for Experimental Pathology [*Federation Proceedings* **32,** 1859–1908 (1973)]. In a discussion following the meeting, the speakers all agreed that no adequate textbook or monograph existed in the fascinating and diverse field of free radical biology. This lack is felt both by workers studying one aspect of the field who would like a broader grasp of other areas and by biologists and physicians who are not working in the field but who wish to learn of recent developments.

The areas included under the general rubric of free radical biology are so varied that no single author could possibly have expertise in all of them. For example, relevant topics include the organic and physicalorganic chemistry of free radicals; the various reactions of oxygen, including autoxidation, reactions of the superoxide radical, and reactions of singlet oxygen; the chemistry of antioxidants, including vitamin E; oxygen toxicity; the chemistry of polyunsaturated fatty acids and their role in membrane chemistry and physics; photochemistry, photobiology, and radiation biology; oxidases, hydroxylating enzymes, and detoxification systems; electron-spin resonance studies of enzymes and substrates, spin-label studies, and esr studies of tissue samples; the toxicity of chlorinated hydrocarbons; the chemistry and biochemistry of smog; the chemistry of cigarette smoke; carcinogenesis of aromatic hydrocarbons, amines, and other compounds; and, finally, the role of free radicals in the time-dependent degradation we call "aging."

In view of the need for an up-to-date review of free radical biology

and the enormous diversity of the areas involved, the participants in the 1970 Atlantic City FASEB meeting agreed that a series of monographs was needed. It has been my pleasure and privilege to serve as editor of these volumes.

I have asked the authors involved in this project to write both for novices and for specialists. I wanted chapters that would not only serve as précis and a "first place to look" for an introduction to a field, but also as up-to-date reviews for experts. This has proved to be a difficult task. So many areas, representing such a diverse background of skills, need to be reviewed that the problem is especially acute. In some cases the subject matter could easily be presented at an elementary level; in others, however, the very nature of the material dictated a more detailed and advanced review. I hope, nonetheless, that most of the chapters in these volumes are at a level that allows them to serve both as a brief introduction to each area and also as an up-to-date survey of each topic.

It seems particularly appropriate that the first of these volumes was published on the two-hundredth anniversary of the discovery of oxygen by Joseph Priestley. Certainly the necessity of organisms tolerating oxygen in their energy-producing systems gives rise to many of the problems and interesting topics in this field. Had glycolysis, or some similar anaerobic process, never been replaced with respiration, organisms would not have had to learn to protect themselves against the oxidative threat that oxygen presents. Also, oxygen appears to be particularly susceptible to one- as well as two-electron transfers, and thus is responsible for producing some of the one-electron intermediates found in the cell.

I hope that these volumes, which bring together many of the diverse subjects in free radical biology, will make these topics accessible to chemists, biologists, and physicians. I also hope that the reader will agree that this is a fascinating, sometimes controversial, and important field.

William A. Pryor

Preface to Volume V

The chapters in this volume attest to the remarkable explosion in research in free radical biology over the past few years. These chapters cover mechanisms for the generation of free radicals; lipoxygenases; the chemistry and biology of oxy-radicals in mitochrondria, white blood cells, and red blood cells; radical-mediated metabolism of xenobiotics; glutathione peroxidase; and aging.

Free radicals are very reactive and they exist at low concentrations. They are produced in initiation reactions; they then react to form products in propagation reactions in which the number of radicals is conserved. Thus, the way in which free radicals are produced in biological systems is a key step that must be understood if free radical pathology is to be controlled. In Chapter 1, S. D. Aust and B. A. Svingen report mechanisms by which free radicals can be produced in enzymatically promoted lipid peroxidation, generally in microsomes or microsomal lipids. They review studies both from Aust's laboratory and from other laboratories, and the data are extremely complex. Aust and Svingen propose that lipid peroxidation can be initiated not only by what has come to be called the "iron-catalyzed Haber–Weiss" system, but also by an initiation process involving an oxidized, ADP-complexed iron ion (which they call the perferyl ion) that is able to convert lipids to lipid hydroperoxides.

In Chapter 2, J. F. G. Vliegenthart and G. A. Veldink report research both from their own laboratory and from others on the biochemistry and biology of lipoxygenases. Less than a decade ago, it was thought that lipoxygenase activity was limited to plants; it is now known, however,

that important lipoxygenase activity occurs in animal cells. In particular, the hydroperoxides of arachidonic acid (such as 5-hydroperoxyeicosatetraeneoic acid) have very potent biological effects. Vliegenthart and Veldink review both animal and plant lipoxygenases, but concentrate most heavily on recent developments in the biochemistry of the classically known plant lipoxygenases.

Two co-workers of Britton Chance review the production of superoxide and hydrogen peroxide in mitochondria in Chapter 3. H. J. Forman and A. Boveris present a historical overview of the field and give detailed information on the methods for detecting and measuring hydrogen peroxide and superoxide. They then review the evidence for the production of these two species from mitochondria in detail. Finally, they consider the biological role of these reactive species in mitochondria and related systems and suggest that while these species may cause pathological changes, their generation also probably serves a physiological role.

In Chapter 4, R. L. Baehner, L. A. Boxer, and L. M. Ingraham discuss the effects of superoxide production in white blood cells, concentrating on an evaluation of the oxygen-dependent reactions of the important phagocytic cells, the monocytes and the polymorphonuclear leukocytes (PMN). During phagocytosis, PMN undergo a rapid oxygen-consuming reaction known as the "metabolic burst"; in this period, superoxide and hydrogen peroxide are generated at relatively high concentrations both within the PMN and in the extracellular environment. It is clear that oxy-radicals play a role in the phagocytic process, although the detailed chemistry is not yet known. Baehner *et al.* make quite clear that the continued study of the role of free radicals in phagocytosis appears certain to provide clues for important advances in therapy.

In Chapter 5, D. Chiu, B. Lubin, and S. B. Shohet discuss the formation and role of oxy-radicals in the red blood cell. Approximately 3% of the circulating hemoglobin in normal individuals is converted to methemoglobin each day; this process continuously generates superoxide within the red blood cell. Although all of the effects of this superoxide are not known, a great deal of information is available. The red blood cell membrane contains a high concentration of polyunsaturated fatty acids (PUFA), and superoxide initiates the autoxidation of PUFA; this results in a decrease in the deformability of these membranes, in turn producing more rapid sequestering of the red blood cells by the spleen. The red blood cell is a classical system for studying the effects of oxy-radicals on biological membranes; the well-known assay for vitamin E status involving the time for red blood cell hemolysis is an application of this technology. The red blood cell also provides a very useful system for studying the protection of biological tissue against radical-mediated

damage, and Chiu *et al.* provide a detailed description of these systems. These authors also review the effects of vitamin E deficiency, glutathione peroxidase deficiency, and other conditions that lead to abnormal red blood cell chemistry. It is becoming clear that peroxidation may be an important factor in such diseases as sickle cell anemia, erythropoietic protoporphyria, and other diseases. Some of these conditions have already yielded to therapy with vitamin E and other antioxidants.

In Chapter 6, R. P. Mason presents a comprehensive review of the production of free radicals during the metabolism of xenobiotics. Environmental toxins can produce free radicals by at least four different mechanisms.

1. Some toxins are themselves free radicals, and therefore, obviously, act by free radical processes. An important example is the nitrogen dioxide radical, present both in smog and cigarette smoke.
2. Some toxins, while not free radicals themselves, are so reactive that they cause free radicals to be formed in tissue. An example is ozone, which is the most important oxidant in photochemical smog.
3. Some toxins are spontaneously autoxidized and lead to the production of superoxide. A number of hydroquinones, some of which are used in cancer chemotherapy, are of this type.
4. Finally, a number of toxins are able to divert electrons from normal electron transport in the cell, producing one-electron intermediates that either are toxic themselves or that lead to the production of superoxide.

R. P. Mason's chapter reviews a variety of toxins that operate by one or more of these mechanisms. It is becoming clear that some of the most troublesome pollutants in the biosphere cause their effects by radical-mediated reactions.

Glutathione peroxidase is a key enzyme that provides protection against hydrogen peroxide and lipid hydroperoxides. Since these species are critical precursors of free radicals, the control of their levels is an important function in the cell. In Chapter 7, L. Flohé gives a broad view of the enzymology, biological functions, and free radical chemistry of this critical protective enzyme. He also summarizes a series of questions that are being probed by current research.

Denham Harman was the first to show that the mean life span of mice is extended by dietary antioxidants. The "free-radical theory of aging" postulates that one of the causes of aging is the accumulation of errors in critical biopolymer molecules over time, and that free radical reactions are one of the mechanisms by which these errors are produced. Thus, antioxidants might retard these error-producing reactions and extend life span. Experiments such as Harman's show that antioxidants do lengthen

the mean life span of mammals, although they do not affect the maximum life span. This seems reasonable, since antioxidants cannot affect the genetically programmed life span of a species; they can only control the rate at which cellular damage occurs by radical mechanisms. In Chapter 8, Harman reviews a number of gerontological principles and then discusses the evidence that antioxidants affect aging. He also discusses a number of important diseases in which free radicals may be involved, including cancer and cardiovascular diseases.

The rapid development of free radical biology is clear from the increasing clinical orientation of many of the chapters in the current volume of this series. It is increasingly clear that radicals play a critical role in important biological functions in the cell, and that the control of radical reactivity will be an important therapeutic tool.

William A. Pryor

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