Radiation Biochemistry

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FOREWORD

A comprehensive review of the broad field of radiation biochemistry such as is presented in this treatise is long overdue. Studies of radiation biochemistry on the molecular and cellular level are now in vogue, due, in part, to new techniques, such as mammalian cell cultures and gradient centrifugation, now available for this type of experimentation. The intensive pursuit of studies in this field has led to discoveries that had only been dreamed of fifteen years ago. Although biochemical changes in tissues and body fluids of irradiated individuals are the sum of the molecular and cellular events throughout the body, there has been little effort to integrate these two aspects of radiation biochemistry or to explain the former changes in terms of the latter. As a result, interest in radiation biochemistry on the body and tissue level has declined in favor of the more basic cellular and molecular radiation biochemistry. This is illustrated by the relatively few biochemists now working on the gross metabolic phenomena caused by radiation exposure.

The main purpose of this work is to review critically the field of radiation biochemistry at all levels and to explain the observations at the body and tissue levels in terms of what is happening at the molecular and cellular levels. It is hoped that such an integrated approach will encourage interest in gross biochemical changes following irradiation. It is hoped also that the discussions in the text and the simplified schematic representation of gross biochemical changes may clarify the complex and sometimes contradictory reports in the literature. And, finally, it is my personal hope that repeated reference to possible biochemical indicators of radiation injury may stimulate work in this

field and lead to the discovery of a simple, yet accurate, practical measure of acute radiation injury in man.

Since the biochemistry of the metabolic changes induced by radiation exposure is a complex subject fraught with many pitfalls, this treatise should be of enormous help to life scientists who are just embarking in the field of radiation biology. In particular, the discussions of the complications introduced by body changes secondary to radiation damage, such as partial starvation and changes in cell populations of a given tissue, should aid in avoiding errors in interpretation that have been committed in the past. Hopefully this will encourage more scientists to enter the field of radiation biochemistry.

This book represents an enormous investment of time and energy on the part of the authors. I trust that other readers will find it as useful as I have.

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PREFACE

In our opinion, radiation biochemistry has three major aims: to describe radiobiological effects in biochemical terms; to elucidate the mechanisms underlying these effects; and to shed light on general biological principles in living organisms. In this two-volume treatise, we have tried to fulfill these aims to the best of our ability.

Because of space limitation, however, we have focused on the radiation biochemistry of mammalian systems, and cite only selected publications, a practice which is certain to result in the unjust omission of many important investigations. We have made a special effort to avoid mere descriptions of radiation-induced biochemical changes and have sought to seek correlations between the various biochemical changes and to interpret radiobiological effects at all levels in biochemical terms.

Volume I, written by Shigefumi Okada, shows the interplay among radiation chemistry, radiation biochemistry, radiation biophysics, and radiobiology which has brought us to our present understanding of cellular radiobiological effects in terms of molecules. The important feature of the interplay is the eradication of the demarcation lines between the disciplines of physics, chemistry, and biology. What excites many radiobiologists is the fact that the concepts developed through intensive radiobiological studies have had great bearing on the life sciences in general. These concepts involve hydrated electrons, DNA repair, artificially induced mutation, cell cycle, cell proliferation kinetics, and organ transplantation.

Volume II, written by Georg B. Gerber and Kurt I. Altman, deals with the

radiation biochemistry of mammalian organs and body fluids. Emphasis is placed on descriptions of overall biochemical changes in irradiated tissues and animals, on the dependency of these changes on cellular responses, and on the interactions among different organ systems. Consideration is also given to a practical application of radiation biochemistry to the problem of assessing the nature, tissue localization, and extent of radiation injury in man and animals.

It is hoped that this work will be useful to radiobiologists at all stages of sophistication as well as to other scientists who are interested in radiobiology. It is also hoped that it will illustrate a unique facet of radiobiology in which one type of environmental insult to mankind has been studied at all levels, individually and integrated, i.e., molecular, cellular, tissue, and whole body. This may serve as a guideline for future studies of many other types of environmental insults besides ionizing radiation. We sincerely hope that readers will sense our feeling of excitement about radiation biochemistry.

S. Okada would like to thank Dr. L. H. Hempelmann for his encouragement and for the many hours he spent in reviewing the manuscript; Mrs. T. Brady for her cheerfulness and tremendous effort in typing, assembling, and checking of the manuscript; and Mrs. Y. Doida for drawing the charts in Volume I. He also thanks Drs. J. N. Stannard, F. Hutchinson, P. Reisz, S. Person, D. Billen, L. J. Tolmach, W. Szybalski, W. C. Dewey, D. K. Myers, M. W. Miller, and J. S. Wiberg for reviewing various parts of the manuscript and for their useful suggestions. Dr. Okada is grateful to the University of Rochester School of Medicine and Dentistry and to the U.S. Atomic Energy Commission [AT-(30-1)-1286 and W-7401-eng-49] for supporting his research through which the concept of this work was conceived and developed.

G. B. Gerber and K. I. Altman are indebted to many, in the United States and Europe, for their assistance at various stages in the preparation of Volume II, particularly to L. H. Hempelmann for his tireless effort in reading and criticizing all parts of this manuscript and for his often-sought advice. They are also grateful to Mrs. I. S. Ytreberg, Mr. Van Gerven, Mrs. J. Remy-Defraigne, and Mrs. K. I. Altman for typing various parts of the manuscript and to the latter also for invaluable help in editorial and stylistic matters; to Mrs. Ytreberg, Mrs. Remy-Defraigne, and Mrs. M. J. Soffer for rendering assistance in searching and collecting literature, a task in which one of us (G. B. Gerber) received much help from CID-Euratom (computerized information storage and retrieval system); to L. Schwartz, J. P. Cooper, and F. Geussens for the preparation of illustrations; to many colleagues for reading and critically discussing various parts of the manuscript among whom are Drs. Z. M. Bacq, J. F. Scaife, C. Streffer, M. F. Sullivan, R. Goutier, A. Sassen, A. M. Reuter, H. Hilz, A. Rothstein, L. L. Miller, J. N. Stannard, W. Staib, J. Hugon, J. R. Maisin, and L. G. Lajtha; and to Christine Mertz and Catherine

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We are also grateful to the staff of Academic Press for their aid and cooperation in the production of this work.

October, 1969

KURT I. ALTMAN
GEORG B. GERBER
SHIGEFUMI OKADA

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TISSUES AND BODY FLUIDS

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Chapter I

RADIATION EFFECTS ON MOLECULES

All radiobiological phenomena in living cells are initiated by the interaction of radiation with molecules. How radiation interacts with molecules and how information on the interaction helps us to understand molecular mechanisms of radiobiological phenomena are the subjects of this chapter.

By 1946, radiobiologists established the existence of two types of action: direct and indirect. Direct action is the interaction of radiation with molecules resulting in damage to the molecules. The concept of direct action came about as a result of the development of "target" theory (*11*). Indirect action is the reaction between solute molecules and "activated" water molecules in aqueous solution. The "activated" water molecules are formed as a result of direct action of radiation with water molecules. The concept of indirect action resulted from studies of radiation-induced chemical reactions in aqueous solution (*11*).

In the next 15 years, a great effort was made to obtain a better understanding of direct and indirect action. The concept of direct and indirect action, expressed in the simplest molecular model of a cell, is shown in Fig. I-1. Since living cells are far more complex than this model, the concept of direct and indirect action as applied to living cells is expected to have considerable limitations.

Since most of the work has been carried out with x-rays and γ-rays, the radiations referred to in this book are generally electromagnetic radiations of high energy (over 200 kVp), unless otherwise specified. As photons of x-rays and γ-rays interact with molecules in a cell, they emit photoelectrons and Compton electrons. These electrons, in turn, interact with molecules which

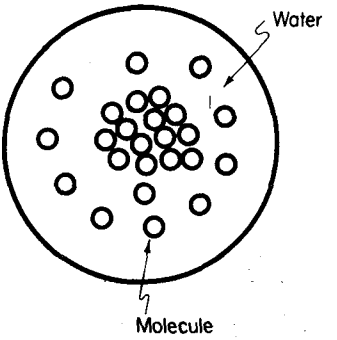

FIG. I-1. The simplest molecular model of a living cell.

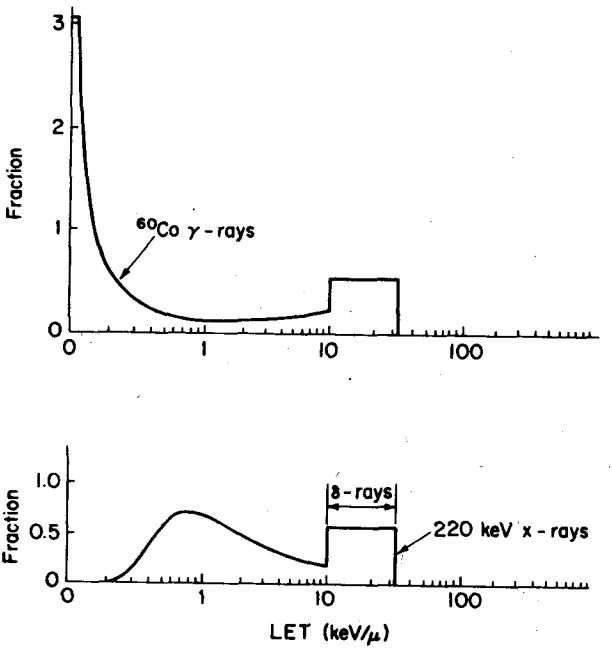

FIG. I-2. LET spectrum in water for x-rays and ^{60}Co γ-rays. The fraction of energy dissipated per unit interval of λ is plotted as a function of λ on a linear scale where $\lambda = \log_{10}$ LET. For convenience, the horizontal scale is also marked directly in LET (keV/μ) in water on a logarithmic scale. The fraction of energy dissipated between any two LET values is equal to the area under the curves between these two limits. The rectangular areas represent the fraction of energy dissipated by δ-rays of 1000 eV and less. The LET values for the core of the tracks of δ-rays of 100 eV and over are considered separately. These spectra apply as long as the track segments (t) are shorter than 100 Å. [From P. Howard-Flanders, *Advan. Biol. Med. Phys.* **6**, 553 (1958).]

then emit secondary electrons. The energy of secondary electrons varies a great deal, and the fraction of electrons with less than a few keV is called "δ-rays," as it produces a short column of dense ionization. One way to express "energy distribution of secondary electrons" is by use of LET (linear energy transfer), which is the energy dissipated per unit length along tracks of electrons. The distribution of LET of electrons from x-rays and γ-rays shows that LET varies by a factor of 100, ranging from LET of several tenths to several tens of keV/μ (Fig. I-2). Since radiobiological effects are dependent on LET (*17a*), the distribution of LET of secondary electrons must be kept in mind.

Before starting discussion of direct and indirect action, let us define D_{37} dose and G value which are necessary for quantitative expression of radiosensitivities of molecules. G Value (often referred to as G) is the number of molecules damaged or of molecules formed per 100 eV of absorbed energy (see Appendix 1 for further information), and D_{37} dose (often referred to as D_{37}) is the dose which damages 63% of the molecules (leaving 37% of the molecules undamaged) (see Appendix 2).

A. Direct Action

Direct action is the interaction of radiation with molecules, thereby inflicting damage to the molecules. Direct action consists of the following three steps.

1. Energy Absorption

The interaction of radiation (or, more correctly, secondary electrons produced by irradiation) with molecules results in transfer of part of the electrons'

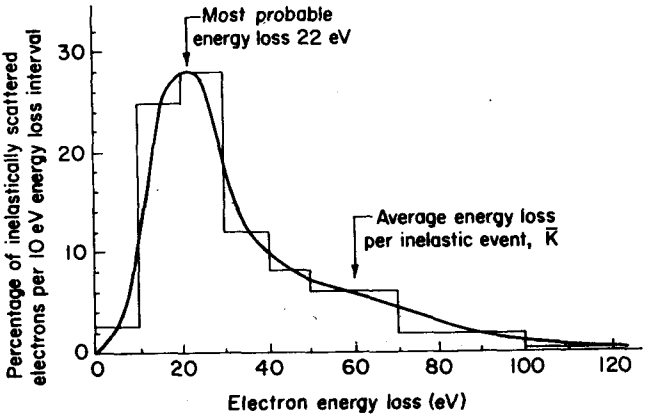

FIG. I-3. The distribution curve of the "first-collision" energy loss for 20 keV electrons passing through a thin layer of Formvar. The experimental data for the 130 Å Formvar was used to calculate the histograms through which the smooth curve is drawn. [From A. M. Rauth and J. A. Simpson, *Radiation Res.* **22**, 643 (1964).]

kinetic energy to the molecule. The amount of energy deposited within molecules per interaction can be estimated by measuring the energy loss of the electrons as they pass through a very thin film of polymer, such as Formvar, polystyrene, and carbon (*14*). The energy loss ranges from zero to about 120 eV, with 22 eV the most probable energy loss and with 60 eV the average energy loss, regardless of the incident energies (5 keV–20 keV) of electrons (Fig. I-3).

The absorbed energy in a molecule can eject one or more electrons from the molecule (ionization) or can raise electrons from a ground state to a higher energy level (excitation). In some instances, the energy needed for excitation exceeds that required for ionization and results in an energy state called "superexcitation" (*2*, *13*).

2. Energy Transfer Processes of Direct Action

Ionized, excited, and superexcited molecules are unstable. In order to form stable molecules, the electron configurations in the molecules must undergo rearrangement within the molecule or through interaction with other molecules. Rearrangement reactions result in dissociation of a molecule into two radicals; in ionization, or in different types of excitation. The reactions occurring between the primary event and formation of final, stable molecules are called the "energy transfer" processes of direct action. It should be emphasized that the energy transfer process of direct action consists of not one, but probably many types of reactions, such as migration of small radicals and migration of excited energy. To express such diversity of the energy transfer processes in direct action, the use of the term "reactivity transfer" has been suggested by Charlesby (*3a*). Experiments of the following types have proved the formation of metastable states in biologically interesting molecules during the energy transfer processes.

a. *Electron Spin Resonance* (*3*, *17*, *21*)

In molecules (e.g., proteins), the composite atoms C, H, O, N, S, and P are held together by chemical bonds, each of which is composed of one pair of electrons (Fig. I-4). In such bonds, each electron has a magnetic spin which is equal to and opposite in direction to the other electron of the pair. Thus, one pair of electrons has a net magnetic field of zero. When such a molecule is irradiated, the molecule often dissociates into two fragments, each of which has one unpaired electron of the previous electron pair. The fragment with one unpaired electron is called a "free radical" or "radical." Since the magnetic spin of the unpaired electron is not canceled, the magnetic field associated with this electron can be detected by means of an electron spin resonance apparatus. Since such an electron can be influenced by other magnetic fields (spins) of the

molecule (such as the nuclei of nearby hydrogen atoms, as well as other electrons), electron spin resonance (ESR) patterns of irradiated molecules are affected a great deal depending on the molecular environment (Fig. I-4).

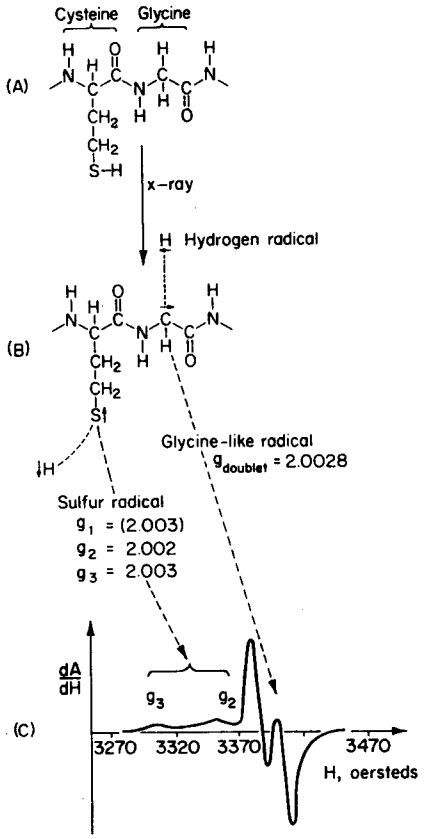

FIG. I-4. Schematic diagram of free radicals in irradiated proteins at room temperature. A. Polypeptide chains before irradiation; B. polypeptide chains after irradiation (arrows are electron spins); C. ESR spectrum of irradiated protein (trypsin) at 295°K. Trypsin was irradiated in vacuum, at room temperature with 6.5 MeV electrons, and the first derivative of the absorption spectrum is given. [From T. Henriksen, *in* "Electron Spin Resonance and the Effects of Radiation on Biological Systems" (W. Snipes, ed.), Nucl. Sci. Ser. Rept. No. 43, p. 81. Natl. Acad. Sci., Washington, D.C., 1966.]

ESR patterns of irradiated molecules can be altered by temperature or by introducing other molecules, e.g., oxygen, hydrogen sulfide, water, etc. Irradiation of a mixture of two types of molecules often results in an ESR pattern which is different from a composite ESR pattern of the two component