CRC

RADIATION HISTOPATHOLOGY

Volume I

George W. Casarett

CRC PRESS

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This book is based on work performed under contract with the U. S. Department of Energy at the University of Rochester, Department of Radiation Biology and Biophysics.

CRC Press, Inc.
Boca Raton, Florida

Library of Congress Cataloging in Publication Data

Main entry under title:

Radiation histopathology.

Bibliography: p.
Includes indexes.
1. Radiation—Toxicology. 2. Ionizing radiation—Physiological effect. 3. Histology, Pathological. I. Title. [DNLM: 1. Histology. 2. Pathology. 3. Radiation injuries. 4. Radiation, Ionizing—Adverse effects. WN610.3 C335r]
RA1231.R2C37 616.9'897'07 79-16204
ISBN 0-8493-5357-2 (Volume I)
ISBN 0-8493-5358-0 (Volume II)

Direct all inquiries to CRC Press, 2000 N.W. 24th Street, Boca Raton, Florida, 33431.

International Standard Book Number 0-8493-5357-2 (Volume I)
International Standard Book Number 0-8493-5358-0 (Volume II)

Library of Congress Card Number 79-16204
Printed in the United States

PREFACE

Radiation histopathology may be defined here broadly as that branch of radiation biology which is concerned with the microscopic study of organized tissue and organ changes caused in vivo by radiation directly and/or indirectly. This book is concerned only with the effects of ionizing radiations on normal tissues and organs as observed by means of light microscopy.

Radiohistopathologic effects represent the consequences of cellular radiobiologic effects and constitute the bridge between cellular radiobiologic effects and gross pathologic and clinicopathologic consequences in the sequences of pathogenetic mechanisms of these gross effects and consequences. Although some biophysical and cellular radiobiologic background is provided, this is neither a cellular radiobiologic nor a clinical radiation pathology book, and is not intended to be a comprehensive treatise of the many and complex interdisciplinary facets of either of these fields. Nor is this book intended to be either an atlas, manual, or comprehensive reference source for radiation histopathology, but rather a treatment of this subject in pathogenetic terms with illustration of the principal features.

This book describes and illustrates the histopathology of ionizing radiations and discusses the pathogeneses of the major somatic degenerative, nontumorous, nonteratogenic effects of ionizing radiations. From the practical point of view, the principal thrust of the book is directed at those changes capable of developing into functional and/or structural impairments or lesions that may cause significant detriment in body systems and adversely affect health. The main focus of the book is the attempt to express radiohistopathologic observations in terms of the cellular dynamics of tissues and organs and to consider radiation histopathology in the general context of tissue response to injury rather than merely the special result of a specific type of injury or agent. As such, the treatment of the subject in this book is primarily concerned with general sequences, pathogeneses, and mechanisms, rather than with great detail and anecdotal material.

By means of reasonably broad classifications and generalizations, this book represents and consistently applies interpretations, theories, concepts, and schemes of relative radiosensitivity and of direct and indirect mechanisms of radiation damage of cells, tissues, organs, and systems in vivo which appear to be highly compatible with the experimental and clinical evidence and which permit reasonable prediction of radiopathologic damage. Permanent, progressive, and delayed radiation effects are considered as well as acute effects. The additivity of these radiation effects with other changes occurring with time or increasing age and the influence of this additivity on the time of appearance and degree of pathologic expression of the radiation injury are also stressed.

Most of the information on which these concepts and interpretations of pathogenetic sequences and mechanisms are based has come from extensive experimentation, with a lesser amount from occasional well-designed extensive studies of human organs and consideration of the aggregate of great numbers of more anecdotal human case reports. Disagreement with some of the author's interpretations, concepts, or criteria is expected in such an interdisciplinary, multilevel field of investigation as that of radiobiology in which there are still so many large gaps in knowledge. The gap between basic cellular radiobiology and clinical radiopathology is still very large. For purposes of study of pathogenetic sequences and mechanisms, there is more valuable radiohistopathologic information, and illustration, for some organs, which have been studied intensively for this purpose, than for other organs. For this reason, certain organs and tissues have been given more or less consideration and illustration in this book.

The illustrative materials used in this book are largely those developed from the research of, and for graduate teaching by, the author in the field of radiation histopathology over the past thirty years. The literature listed as being cited specifically and/or as other sources omits of necessity many of the vast numbers of publications which have more or less bearing on the subject. The references and other sources include those articles and books used in the review of specific subject matter. It is from this diversity of selected background information that the text of this book has been derived.

The author hopes that this book will help the reader, whether a researcher or clinician, to understand better the translation of cellular effects of ionizing radiation into subsequent detriment in the body.

George W. Casarett, Ph.D.

ACKNOWLEDGMENTS

The author wishes to express his gratitude to all of those people who have contributed to the experience on which this book is based: to research scientists in this field, past and present; to his collaborators in research over the past three decades; to the many graduate students, who have contributed much of the information used in the book; and to his technical associates who have contributed vitally to his research.

Many thanks are owing to Beverly Holloway for her excellent secretarial and editorial capability in the production of this manuscript.

Most of all, the author wishes to convey his deepest gratitude to his wife, Marion, and daughter, Vicki, for their many kinds of vital support and encouragement.

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THE AUTHOR

George W. Casarett, PH.D. is Professor of Radiation Biology and Biophysics, and of Radiology at the University of Rochester School of Medicine and Dentistry, Rochester, New York.

Dr. Casarett obtained his premedical education at the University of Toronto (1938-1941) and the University of Rochester (1943-1945). He undertook his Graduate studies at the University of Rochester where he earned his Ph.D. in Anatomy (Pathology) in 1952. Since 1943, Dr. Casarett has held numerous Research and Academic positions at the University of Rochester School of Medicine.

Dr. Casarett is currently Chairman of Scientific Committees #1 and #14 of the National Council on Radiation Protection and Measurements, of which he has been a Director. He is a recent past Chairman of the National Academy of Sciences Advisory Committee on Biological Effects of Ionizing Radiation.

Among his numerous past activities in national and international organizations, Dr. Casarett has been a member of the National Academy of Sciences Advisory Committees to the Federal Radiation Council and to the Atomic Bomb Casualty Commission, the National Cancer Institute Research Training Committee, and Committee #1 of the International Commission on Radiological Protection, and a consultant to the United Nations Scientific Committee on Effects of Atomic Radiation and the Nuclear Regulatory Commission. His membership and Fellowship in numerous scientific societies include memberships in the American Association of Pathologists, Radiation Research Society (former councillor and associate editor of journal), American Association of Anatomists, and Society for Experimental Biology and Medicine.

Dr. Casarett has authored over 200 papers on various aspects of radiation research. His major fields of research are in Radiation Pathology, Radiation Biology, Carcinogenesis, Gerontology, and Cancer Biology.

DEDICATION

For Marion

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Chapter 1

GENERAL RADIATION BIOPHYSICS AND BIOLOGY

This chapter is intended to provide briefly and generally some background information useful in consideration of the subjects dealt with in subsequent chapters.

I. RADIATIONS

Energy from radiations of relatively long wavelength, e.g., in the ultraviolet range, may be dissipated in matter largely in the process called excitation in which an electron is raised to a higher energy level. The energy of radiations of shorter wavelength may be transferred by ionization as well as excitation, ionization involving the removal of an electron from its atom or molecule.

This book is concerned primarily with biophysical events which are the consequences of the interaction of ionizing radiations in matter, particularly living matter.

X-rays and gamma rays are electromagnetic radiations consisting of streams of energetic photons which can cause ionization. The peak energy and degree of penetration of the radiation beam generated by a particular X-ray tube, determined by its characteristic wavelength, is described in terms of kilovoltage peak, kVp. Gamma rays have a single energy because they result from discrete nuclear disintegrations and are generally highly penetrating. In all other respects, X-rays and gamma rays have roughly similar properties in terms of their interaction with matter. Their paths in matter are relatively straight.

Electrons, or beta particles in the context of radioactive disintegrations, are particles of very small mass carrying a single negative charge. As electrons are easily deflected from their paths by other electrons, their tracks in matter are tortuous and the depth to which they penetrate matter is shorter than the total track length. However, the specific ionization or density of ionization is greatest near the end of the track, because of the increasing probability of interaction between the electrons and atoms of the target material at the lower velocity. The penetration of matter by electrons varies directly with their energy, e.g., being greater for electron beams from a megavoltage betatron or a linear accelerator than for the 0.53 meV beta particles from a strontium-90 source (1 or 2 mm in tissue).

Alpha particles are relatively large particles (8000 times heavier than electrons) carrying a double positive charge. They are helium nuclei (two protons and two neutrons) lacking two electrons. Their velocity is much lower than that of electrons, they penetrate tissue very shallowly, a few hundred μm at most (depending on the tissue density), their tracks are straight, and the ionization density is greatest near the end of the track. Because of the shallow penetration, alpha radiation is relatively unimportant as an external source of radiation, but it is important as a radiation emitted from various radionuclides deposited within the body. Because of their low velocity and penetration and their double charge, alpha particles are very densely ionizing. In terms of linear energy transfer (LET) units (kev per μm of track length), the average value V for alpha particles would be about 100, as compared with 3.0 for orthovoltage X-rays and 0.3 for gamma rays.

Neutrons are noncharged particles which produce no ionization directly, but interact with matter by direct collisions with atomic nuclei. Slow or thermal neutrons are captured upon entering atomic nuclei, while fast neutrons (energies greater than 20 keV) interact mainly by elastic collisions with the nuclei. In terms of transfer of energy, the most efficient interaction is that involving direct collision with a proton (hydrogen

nucleus), a particle equal in mass to that of a neutron, such that up to all of the neutron energy may be transferred to the proton, which recoils. As there is a high concentration of hydrogen atoms in most tissues, a fast neutron beam produces many recoil protons, heavy positively charged particles which lose velocity quickly in tissues and become very densely ionizing near the end of the straight track (LET up to 90 keV/μm) in a manner similar to that described above for alpha particles, and with an average LET of 10 to 50 keV/μm, depending on the energy of the neutrons.

Collisions between fast neutrons and nuclei of oxygen, carbon, nitrogen, and other atoms can also produce ionizing recoil particles. Also, inelastic scattering of fast neutrons may result from interaction with heavier atomic nuclei in tissue, with the production of gamma rays. Still other forms of neutron energy transfer include neutron capture and nuclear disintegration. Neutron capture by hydrogen or nitrogen atoms makes these atoms radioactive, with the emission of gamma rays and protons, respectively. When neutron absorption into nuclei results in great instability, the nuclei may explode, with the emission of alpha particles, deuterons, protons, and other neutrons.

Heavy charged particles may also be accelerated by high energy machines, e.g., a synchrotron, so that such densely ionizing particles may be energetic enough to penetrate tissue to useful depths, as in radiotherapy.

The energy of electromagnetic radiations is transferred to matter by one or more of three mechanisms, depending upon the energy spectrum of the radiation. For energies less than 0.5 meV, the main mechanism is the photoelectric effect in which the photon is completely absorbed by the target atom, an electron is emitted, and characteristic radiation is produced. For megavoltage X-rays and gamma ray sources with a peak energy greater than 1 meV, the main mechanisms are Compton scattering and pair production. Compton scattering is predominant over the energy range of 0.5 meV to about 5 meV. The incident photon collides with a planetary electron, produces a recoil electron, leaves with diminished energy, and then, depending upon its energy, may interact with additional target atoms by Compton scattering or the photoelectric effect. Pair production begins at photon energies above 1.02 meV, but starts to become important quantitatively with megavoltage X or gamma radiations above 20 meV peak energy. The incident photon is converted into an electron and a positron, the positron being a positively charged electron which is eventually annihilated by collision with a negative electron to produce two photons of 0.51 meV energy.

Ionization involves dissipation of an average of 34 electron volts when it occurs in air, and probably less in liquids and solids. About two-thirds of this energy is dissipated in excitation, which is relatively unimportant biologically, and the remainder in ionization. With the ejection of an electron from an outer shell of an atom, the atom becomes positively charged (positive ion) and the ejected electron may then interact with another atom to form a negative ion to complete the production of an ion pair. The lifetime of such an ion pair is very short (of the order of 10^{-10} second) before they result in relatively more stable neutral free radicals (lifetime up to 10^{-3} second).

II. BIOPHYSICAL ASPECTS

About half of the biological effect of radiation is the indirect result of free radicals produced in water, and the other half is due to direct ionization of critical biological molecules.

Although many molecules in aqueous solutions already exist, at least partly, in ionized states owing to simple dissociation into positive and negative ions in stable equilibrium, irradiation produces pairs of abnormal ions (free radical ions) which are not in equilibrium. Water is ionized by irradiation into H_2O^+ ions and free electrons. Such free radical ions are extremely unstable and form neutral free radicals, i.e., uncharged

atoms or molecules with an unpaired electron in the outer orbit. Both free radical ions and the resultant free radicals disrupt normal molecular structures and damage biological targets.

Thus following the primary events in the absorption of radiation energy in tissues, a complex series of events leads to the breakage of various chemical bonds. As living material consists of 70 to 90% water, the radiolysis of water is of great importance in the production of biological effects. The primary event is the ionization of the water molecule to produce a positive ion and a free electron. This is followed by a complex series of reactions leading to various chemical products. Biologically it makes little difference whether a molecule is damaged directly or indirectly through ionization of water.

The relative importance of these primary direct and indirect effects at the cellular level varies with the LET of the radiation, i.e., the distribution of ionizing events in relation to the target and the probability of these ionizations occurring inside or outside a critical target volume for specific biological effects. A biological target in a cell is more likely to receive the damage required for a biological effect by the direct mechanism with high LET radiation and by the indirect mechanism with low LET radiation. The high LET radiation will also be more damaging, dose for dose, i.e., will have a greater relative biological effectiveness (RBE).

The densely ionizing radiations (high LET) have a greater probability of hitting an intracellular target more than once than the sparsely ionizing radiations (low LET). As seems to be the case, if the production of significant biological damage in a complex target, such as the cell, requires that some critical or sensitive part of the cell be hit more than once or that two or more sensitive sites be hit, high LET radiations are more likely to satisfy such requirements.

Rossi[19,20] and Kellerer and Rossi,[13,14] on the basis of their observations that the RBE of neutrons for different biological effects varies inversely as the square root of the dose over a wide range of doses, have theorized that this represents a general radiobiological relationship. They postulated that the biological effect is the result of changes or impairments at two sites within a single target in the cell nucleus, and that the yield of such changes for a given LET is proportional to the mean square of the specific energy (absorbed energy at a site divided by the mass of the site) and thereby proportional to a quadratic function of the absorbed dose. In other words, the yield (Y) of impairments is a function of the proportional dose (D) term plus a dose squared relationship term as in $Y = aD + bD^2$, where the coefficient a is a function of the size of the site and the radiation quality (LET), and the value of the coefficient b varies from 0 to 1, depending on the probability of interaction between sites of radiation impairment and therefore on dose rate and time available for repair. For high LET radiations the linear term greatly predominates, and the dose square term is of little importance over the practical range of doses. For low LET radiation the dose square term is important at high dose levels, and the linear term becomes important only at low dose levels. Spreading out the primary impairments produced by an intermediate or high dose of low LET radiation by reducing the dose rate reduces the probability of interactions between impairments because of repair.

As the effect of high LET radiation is proportional to dose (linear) and independent of dose rate, the RBE of that radiation increases as the dose and/or dose rate decrease; not that the effectiveness of the high LET radiation changes, but because the effectiveness of the low LET reference radiation decreases with reduction of dose and/or dose rate. In regard to any requirement for a minimum amount of radiation energy deposited within a small body to initiate a biological effect, the probability of low LET radiation producing the effect with a single track or hit is small, and the probability increases with increasing LET to a point above which the efficiency in terms of energy

per unit mass may decrease with increase of energy deposition that is superfluous or wasted in terms of production of the specified effect. Thus the RBE may increase with increasing LET to a peak at an intermediate LET, often observed to be from about 100 to 300 keV/μm, and then decrease as LET rises further, without dependence on size of dose and dose rate.

On the assumption that most cellular radiobiological data support a multitarget single-hit theory, if only one sensitive site is hit then the cell may recover from such physical damage and the significant biological effect will not occur. However, if more than one sensitive site is hit, then the biological effect may occur and be irreversible. High LET radiations are more likely to produce the effect than low LET radiations. Thus under comparable conditions and in the practical ranges of dose, high LET radiations are relatively more effective in producing biological damage than low LET radiations. Spreading out the radiation dose in time, i.e., substantially reducing the dose rate, may reduce the effectiveness of low LET radiation because of repair of some injured sites before injury occurs in other sites. For this reason, reduction of dose rate has much less influence on the effectiveness of high LET radiations.

Relative biological effectiveness (RBE) is defined as the ratio of the dose of a standard reference low LET radiation dose to the dose of another radiation required to produce the same effect under given conditions. If one chooses as the standard reference a low LET radiation, a radiation with low effectiveness, such as megavoltage X-rays or ^{60}Co gamma rays, and assigns to such radiation an RBE value of 1, then medium voltage X-rays tend to have a slightly greater RBE value and fast neutrons, alpha particles, and accelerated heavy charged particles, i.e., high LET radiations, tend to have much higher RBE values. However, in the case of the high LET radiations, the RBE values tend to vary considerably with dose size and/or dose rate, as well as with level of biological endpoint, the RBE tending to increase with decreasing dose and/or dose rate. This variation with dose size and dose rate is due to the reduction in effectiveness of the standard reference low LET radiation with decreasing dose and/or dose rate.

The main products of the radiolysis of water are the oxidative OH radicals and the reducing hydrated electrons. Compounds that have an affinity for electrons tend to radiosensitize cells and tissues. As the degree of the initial indirect radiobiological damage from low LET radiation depends to some extent on the competition between dissolved or available oxygen and endogenous hydrogen donors in a tissue, the concentration of oxygen near sensitive biological targets influences to some extent the amount of initial radiation damage. Highly oxygenated tissues tend to sustain greater radiation damage than hypoxic or anoxic tissues, all other conditions being equal. Relative protection or sensitization of tissue to the initial effects of low LET radiation depends upon the balance of reducing and oxidizing substances in the immediate environment of the critical targets. As the LET of the radiation increases, and therefore the degree of direct irreversible damage of targets and the overall degree of damage increases, the influence of oxygen availability decreases.

Protective compounds such as those containing the sulfhydryl (SH) group, e.g., cysteamine and cysteine, may act against the oxygen influence by enabling restoration of ionized molecules to their normal state. Therefore, radiosensitizers other than oxygen act not only by altering the oxidative-reduction balance, but also by intracellular binding of naturally occurring radioprotective substances, such as the SH compounds.

III. RADIATION QUANTITIES AND DOSE

The Roentgen (R) is the unit of exposure for X or gamma radiations and relates to

the quantity of ionization produced in air. It is defined as 2.58×10^{-4} coulomb per kg of dry air.

Until recently, the rad (100 ergs/g tissue) has been the unit of absorbed dose of ionizing radiation, and has been replaced by the gray (gy) which is equal to 100 rads. These units apply to particulate and electromagnetic radiations. As the absorbed dose of X or gamma radiation (100 keV to 3 meV) in water and soft tissues is almost equal numerically to Roentgens, the rad and the Roentgen will sometimes be used interchangeably in this book.

Linear energy transfer (LET) is defined in limited fashion here for the purposes of this book as the mean energy lost per unit length of radiation track and is commonly expressed in terms of keV/μm.

The physical quantities defined above were developed primarily to provide a quantitative physical basis for the assessment of biological effects of ionizing radiation. However, it is now well established that equal absorbed doses of various ionizing radiations of different quality (different LET) can cause different degrees of effect and that such differences may be especially pronounced at low doses and/or dose rates, particularly when high LET radiations are compared with low LET radiations.

The relative biological effectiveness (RBE) of any radiation R, as compared with some standard reference radiation S of low LET, is defined as D_S/D_R, which is the ratio of absorbed doses required for equal effect, all other conditions being equal. RBE is an LET-dependent factor.

Because of the variation of RBE with dose size and/or dose rate, nature of effect, and LET, there have been chosen for practical purposes of radiation protection, single values for effectiveness of various categories of radiation quality (LET), e.g., to facilitate equating or summation of doses from various radiations in terms of effects or risks. These selected values are called "quality factors" (QF or simply Q). For conservative purposes, it has been necessary to select Q values not greatly different from RBE values that apply to various systems at various dose and dose rate levels. Consequently, Q values for high LET radiations are likely to be higher than RBE values found in many radiobiological experiments utilizing relatively high doses and/or dose rates, because of the known increase in RBE of high LET radiations with decreasing dose size and/or dose rate. The use of the RBE unit is now limited to radiobiology.

The Q values currently in use in radiation protection practice are 1 for X rays, gamma rays, and electrons; 10 for neutrons and protons; and 20 for alpha particles and other heavy, multiply-charged particles.

For purposes of radiation protection, it has been convenient to use a quantity, the dose equivalent, which is more predictive than the rad and better correlated with the radiation effects of most concern in radiation protection. The dose equivalent (H) is the absorbed dose (rad) multiplied by the quality factor (Q) and the product (N) of all other specified effect-modifying factors (such as dose rate and dose distribution factor). The value for N is sometimes 1, sometimes greater, and sometimes smaller. The dose equivalent at a point in tissue is described by the equation $H = DQN$.

Until recently, the special name for the dose equivalent unit has been the rem, which has been replaced by the Sievert (Sv) which is equal to 100 rems.

The Curie (Ci) is a unit of radioactivity for radioactive nuclides and is equal to 3.7×10^{10} radioactive disintegrations per second.

IV. RADIOBIOLOGICAL ASPECTS

Mammalian cells are enclosed by a cellular membrane (lipoprotein) and contain a nucleus (except for erythrocytes) bound by a nuclear membrane (except at cell division). The nuclei contains the chromosomes or chromatin substance with its deoxyri-