Radiation Protection In Mammals

JOHN F. THOMSON

Radiation Protection In Mammals

JOHN F. THOMSON

Division of Biological and Medical Research
Argonne National Laboratory
Argonne, Illinois

NEW YORK
REINHOLD PUBLISHING CORPORATION
Chapman & Hall, Ltd., London

Library of Congress Catalog Card Number: 62-16372
Printed in the United States of America

Printed in United States of America by
The Guinn Co., Inc.
New York 14, N. Y.

Preface

Chemical protection against radiation damage is essentially a pharmacologic or toxicologic problem, and the general principles concerned with evaluation of any kind of drug apply equally to those used for radiation protection. Thus, on the bases of (a) a continuing interest in the field for the past dozen years, and (b) experience as a pharmacologist in testing and evaluating several hundred chemicals for noxious, protective, or therapeutic properties in chemical warfare, I feel justified in attempting to write a book on the subject of protection against the effects of ionizing radiation.

This book was originally intended to be a collaborative effort with Dr. Jack Schubert, formerly of Argonne National Laboratory, who was to discuss the mechanisms of protection against damage produced by internally administered radioisotopes and techniques of radioelement removal. Unfortunately, for this collaboration, Dr. Schubert was invited to accept a professorship in South America, and was unable to complete his part of the book. Under these circumstances, it was considered advisable to restrict the scope of the book to the problems of prevention and treatment of damage caused by external irradiation of mammals.

The limitation of this material to protection in mammals should not be construed as a denial of the fundamental importance of many studies on protection of other systems. The work on viruses, single-celled organisms, plants, insects, etc., has contributed significantly to our knowledge of mechanisms both of radiation damage in living systems and of modification of this damage. The recent book edited by A. Hollaender,

"Radiation Protection and Recovery," provides an excellent survey of protection in other systems.

It seems probable that the overwhelming majority of types of compounds that might protect against external radiation are now known; within the past few years there has been a general falling off of the numbers of papers reporting new protective agents in animals. Instead, the emphasis is shifting more and more toward the elucidation of the mechanism by which the recognized protective agents act.

Thus this book is intended to be an extensive and critical review of mammalian protection, a field of radiation biology which may not be of great fundamental importance, but which is certainly of considerable interest. No claim is made for completeness of coverage in this field; much of the information, particularly on chemical protection, is available only in project reports and has not been published in the open literature. With one important exception (the reports from the University of Chicago Air Force Radiation Laboratory), this source of information has been largely ignored. Much of the work done in the Iron Curtain countries has been made available only in the form of reviews, and the opportunities to consult the original papers have been somewhat limited. Thus the bibliographies are considered adequate but not exhaustive. It has not been thought necessary to cite every reference that merely confirms the work of other authors. Specific documentation for the material presented in Chapters 1 to 3 has been largely avoided; for the rest of the book, specific references have generally been cited. When an author has reviewed much of his own work on a particular compound, the reference to the review has been used instead of a large number of individual citations. The survey of the literature was concluded on November 1, 1961.

I am greatly indebted to Drs. Harvey M. Patt and Douglas E. Smith for the time and effort that they have spent in reading the manuscript, and for calling my attention to a number of errors of various sorts. Dr. Jack Schubert also offered some helpful advice on organization of the material and read most of the manuscript in its early stages.

Doubtless there are a number of omissions which some readers may consider important; there may be some errors in fact or interpretation, despite reasonable diligence, for which I can only apologize in advance.

JOHN F. THOMSON

Argonne, Illinois
April, 1962

Contents

1. Introduction

NATURE OF RADIATION

It is beyond the scope of this book to present a detailed account of the physical and chemical aspects of the effects of ionizing radiations. A number of excellent texts are available in which the problems of fundamental radiobiology are discussed. Much of the material that constitutes the discipline of radiobiology—which we can define as the study of the interaction of radiation with biological systems—is not directly relevant to the problems of modifying radiation damage in as complex a system as the mammalian organism. These problems are to a large extent pharmacological rather than radiobiological; a large number of compounds that have been shown to protect animals were tested for no reason, or for the wrong reasons. One should not infer, however, that the prevention and treatment of radiation-induced injury are wholly on an empirical basis. A certain amount of radiation physics and chemistry is thus essential to an understanding of protective mechanisms.

Types of Radiation

Ionizing radiations are conveniently divided into two classes: electromagnetic, including X- and γ-rays; and corpuscular, including β-rays (electrons), α-rays (helium nuclei), protons (hydrogen nuclei), and neutrons. In radiobiology, X-rays, γ-rays, and neutrons represent the most commonly employed external sources of radiation, whereas α-, β-, and γ-radiations associated with the decay of radioactive elements are of primary importance as internal sources.

The energy of X- and γ-rays is almost entirely absorbed

by the ejection of electrons from atoms through which these radiations pass; the atom having lost an electron is said to be ionized. The energy of a quantum of these radiations, except for very soft X-rays, is much greater than that necessary to ionize an atom, and this excess energy is stored in the ejected electron, which in turn can produce ionization in atoms through which it passes.

Perhaps as much as 75 per cent of the total amount of the energy is dissipated as heat without producing ionizations. The temperature rise following doses necessary to kill most mammals is only a few thousandths of a degree.

Depending upon the energy of the radiation, the X- or γ-photons will (a) eject an electron from the innermost shell of electrons (photoelectric effect); (b) eject an electron from an outer shell (Compton effect); or (c) produce an electron and a positron, the latter of which will be annihilated to produce two more photons (pair production).

These electrons, as well as those ejected by β-emitting radioactive elements, in turn collide with bound electrons of atoms, with the result that additional electrons are expelled, some of which may retain enough energy to produce additional ionizations. Ultimately, however, the energy level of the electrons drops below that necessary to produce ionization. A similar mechanism obtains with α-particles and protons. Each corpuscular radiation produces a characteristic track within the medium through which it passes.

Neutrons, which are uncharged particles of approximately the same mass as the hydrogen nucleus, do not produce ionization. Depending upon the energy of the neutron, it can either eject a proton from an atomic nucleus, or be absorbed by the nucleus of an atom, which then becomes radioactive and emits β- and γ-rays. The former process is characteristic of "fast" neutrons, which have been extensively used in radiobiology; relatively little use has been made of "slow" neutrons in radiobiological research.

The extent to which radiation can alter the properties of the medium through which it passes thus depends both on the nature of the particles and on the kinetic energy of each particle. The kinetic energy is usually defined in terms of electron volts (ev);

1 electron volt is equivalent to 1.6×10^{-12} ergs, or 23,000 calories per mole. For radiations of biological significance the energies are usually stated in terms of thousands (kev) or millions (Mev) of electron volts.

Penetration of Radiation

An important difference among heavy charged particles, electrons, and the electromagnetic radiations is in their ability to penetrate matter. Protons and α-particles have extremely short ranges; a 1 Mev proton in tissue will yield all of its energy within a few microns, an α-particle of the same energy in an even shorter distance. Electrons dissipate their energies at lower rates, and thus have greater ranges, although because of the fact that they may be deflected, the linear distance from their point of ejection may be somewhat shorter than the total distance that they travel. In tissue, the range of a 1 Mev electron will be about 0.4 to 0.5 cm. For X- or γ-radiation, interaction occurs with matter at rather widely separated loci. It is not practical to speak of "ranges" in connection with X- or γ-radiation; rather, the degree of attenuation of the radiation dose by the medium through which it passes is employed. Thus, for 1 Mev photons, the half-value layer, i.e., the thickness of tissue required to reduce the incident X- or γ-ray dose by one-half, is about 10 cm.

Linear Energy Transfer

One of the critical factors in establishing the relative biological effectiveness of different types of radiation is the rate at which energy is lost. The older term, "specific ionization," which referred to the number of ion pairs produced per unit length of the track, has been largely supplanted by the expression "linear energy transfer," customarily abbreviated LET, the rate of energy loss per unit length. The LET of a charged particle is directly proportional to the square of its charge and inversely proportional to its velocity; the latter in turn is approximately inversely proportional to its mass. Thus, the LET of an α-particle will be much greater than that of an electron of the same energy, since the former will dissipate its energy

over a very short distance, while the energy loss from the latter will be distributed over a track length about a thousand times longer.

In terms of specific numbers, the LET for β-rays with mean energies above 0.4 Mev will be about 0.25 kev/micron track length. Depending on energies, the LET for X-rays can range from 0.5 kev/micron to about 15. Fast neutrons, depending again on their energies, have LET's between 8 and 43 kev/micron. For α-particles, the maximum LET is about 260 kev/micron. A detailed discussion of LET and its relation to radiobiological effectiveness has been presented by Zirkle.[1] The relationship between LET and the relative biological effectiveness (RBE) of different qualities of radiation is not predictable *a priori*, since different test systems will show different types of responses. In the specific case in which we are primarily interested, acute toxicity to mammals by total-body irradiation, the RBE increases with increasing LET; i.e., fast neutrons are more effective than 200 kv X-rays, which in turn are more effective than Co^{60} γ-radiation, roughly in the ratio 4:1:0.7. This ratio may vary somewhat, depending on the age, sex, strain, and species of animal, the dose rate, and possibly other factors as well.

UNITS OF RADIATION

The most commonly used unit of external radiation (X and γ) is the roentgen, abbreviated *r*. The roentgen is defined as that quantity of X- or γ-radiation such that the associated corpuscular emission per 0.001293 g of air produces, in air, ions carrying one electrostatic unit of quantity of electricity of either sign.

The roentgen can be converted from electrostatic units to energy units, i.e., equivalent to 83 ergs/g. In order to compare particulate radiations with electromagnetic radiations, the "roentgen equivalent physical" or *rep* was introduced, and defined as the amount of energy absorbed per unit mass equal to the energy of one roentgen. Unfortunately there is considerable uncertainty as to the energy absorption in *tissue*; figures ranging from 83 to 95 ergs/g have been suggested.

To avoid ambiguity, a new unit, the *rad*, was defined in 1955

as the amount of radiation producing an absorption of 100 ergs/g. The rad has almost completely superseded the rep for α-, β-, and neutron irradiation, but has not entirely replaced the roentgen for X- or γ-radiation. The roentgen continues to be used for the overwhelming majority of past and present work in protection against ionizing radiation.

Another unit occasionally used is the *rem*, or roentgen equivalent mammal. This unit is the rep (or the roentgen) multiplied by the radiobiological effectiveness (RBE) for the quality of radiation concerned. There seems to be little justification for the use of the rem, since no definite value for RBE can be assigned to cover all effects of a given type of radiation. It is far better to report simply the radiation dose in rads or roentgens, and to indicate *if known* the RBE.

MECHANISM OF ACTION

There are two possible explanations for the effect of radiation on living tissue. One of these is the direct inactivation of some essential component of the cell by ionization, i.e., a direct hit; and the other is the indirect inactivation by an intermediate which is produced by radiation and which then reacts with the critical compound. Simply on the basis of relative frequency of different molecular species in biological systems, the indirect action has been favored, with water molecules as the obvious choice for energy absorption.

It has been estimated that within 10^{-12} seconds after the passage of a particle or photon-induced electron through an aqueous medium, the free radicals H· and OH· are formed. In less than a microsecond, these radicals have either (a) recombined to form water, (b) combined with identical radicals to yield molecular hydrogen and hydrogen peroxide, or (c) reacted with solutes present in the system. A high concentration of solutes decreases the probability of reactions (a) and (b) taking place.

In the presence of oxygen, the hydroperoxyl radical, HO_2·, is formed by interaction between H· and molecular oxygen. Thus the production of oxidizing agents, the free radicals OH· and HO_2· and the compound H_2O_2, is favored over the formation of

the reducing free radical H·. These concepts have been used as a working hypothesis to explain the "oxygen effect" in radiobiology, i.e., the increased sensitivity of most test objects when exposed to X-rays in the presence of oxygen.

The free radicals formed by radiolysis of water are highly unstable, with very short half-lives. How they disturb the physiology of the cell is not fully understood. It has been estimated that free radicals formed in water by radiation can migrate only 20 to 40 Å before reacting. It is still largely a matter of conjecture whether these radicals react directly with vulnerable cellular components to initiate the chain of events leading to manifest radiation injury, or whether they oxidize some intermediate compound, not in itself irreplaceable (e.g., an unsaturated fatty acid) to form a peroxide which is less reactive but more stable, and hence can diffuse farther away from the ionization track to produce the initial biochemical lesion.

For several reasons, the latter possibility is favored, although the former cannot be excluded. Changes occurring after irradiation of solutions of unsaturated fatty acids, nucleic acids, and proteolytic enzymes have been described; unlike the initial photochemical reaction, these changes are influenced by temperature and oxygen tension. Although these observations indicate a possibility of interrupting the sequence of post-irradiation events by prompt introduction of some substance which could compete successfully for the damaging agents, it is probable that irremediable damage is produced within a very short time after ionization occurs.

Evidence has recently been presented which casts doubt on the explanation of the oxygen effect in terms of formation of the hydroperoxyl radical. Work carried out on microorganisms and on human cells in tissue culture has shown that nitric oxide can simulate oxygen in increasing the sensitivity to radiation. Further work must be carried out to explain the augmentative effect of nitric oxide; the situation is complicated by the fact that under certain conditions, e.g., in dry bacterial spores, nitric oxide may *decrease* radiation sensitivity. It may be pointed out, however, that regardless of the mechanism involved, it is sufficient to state simply that in mammalian radiobiology the oxygen effect does exist.

Another mechanism has been suggested by Alper[2] in which direct action plays a predominant part. According to this hypothesis, the energy of the radiation is absorbed by a cellular component; the fate of this ionized compound is then determined by its environment. Thus the activated compound could combine with molecular oxygen and become irreversibly altered; this secondary reaction has been termed "metionic". Conversely, if oxygen were removed, or if the target were modified by extraneous protective compounds, the metionic reaction would not occur, and the restoration of the ionized cellular compound to its "native" state would be favored. Whether this hypothesis is correct or not in all of its details is uncertain; its importance lies in the fact that it provides a plausible explanation for both the oxygen effect and the possibility of chemical protection in terms of the direct action of ionizing radiation.

Evocation of Cellular Dysfunction

The intermediate steps between the initial absorption of energy and the appearance of recognizable changes in living tissues still remain obscure. It is certain that some molecules of the cell must be more susceptible than most to inactivation, either through direct ionization or through destruction by free radicals. It is also certain that many molecular species which are altered by radiation are readily replaceable, so that inactivation of one enzyme in a single cytoplasmic particulate would have negligible influence on the economy of the cell.

Hence it is generally presumed that irreparable damage to the cell can occur only if a unique or irreplaceable molecule is altered. Most probably such an event would occur in the nucleus of the cell, rather than in the cytoplasm; experimental evidence of several types bears out this supposition. It is probable that radiation injures equally both the nucleus and cytoplasm. However, although cytoplasmic damage may augment the injury to the nucleus, the former would seem to be of less importance *per se* for the cell and hence for the organism; cytoplasmic damage is more readily reparable, or at least the cell can compensate for an appreciable impairment of cytoplasmic function. Furthermore, damage to the cytoplasm probably is not trans-

mitted in cell division; this is in contrast to nuclear damage, which may result in permanent genetic change.

Opportunities for Protection and Reversal. Alexander[3] has presented a simplified general scheme of the sequence of events after irradiation, which is presented below with slight modification:

Energy absorption
(a) ↓
Molecules chemically changed
(Initial chemical lesion)
(b) ↓
Biochemical lesion
(c) ↓
Physiological and anatomical lesion
(d) ↓
Death of the organism

Protection can be provided by intervention at many of these steps. Shielding, of course, is the only way to prevent energy absorption. However, the presence of appropriate chemical compounds may affect steps (a) and (b), either by diverting the absorbed energy to the destruction of an extraneous molecule (the protective agent), by providing a means of repair of the initial chemical lesion before a biochemically critical molecule can be altered, or by altering the biochemical target so that it is less readily damaged.

There may be some question as to whether a biochemical lesion can be repaired; it is probable that the anatomical lesions cannot be repaired. However, it is possible under some conditions to *replace* damaged cells and prevent the death of the organism. It is also possible to modify step (d) in such a way that the impact of dysfunction of individual organs on the whole animal is lessened.

References

General

1. Bacq, Z. M., and Alexander, P., "Fundamentals of Radiobiology," 1st ed., New York, Academic Press, 1955; 2nd ed., New York, Pergamon Press, 1961.
2. Claus, W. D., ed., "Radiation Biology and Medicine," Reading, Mass., Addison-Wesley, 1958.
3. Ellinger, F. P., "Medical Radiation Biology," Springfield, Ill., Charles C Thomas, 1957.
4. Errera, M., and Forssberg, A., eds., "Mechanisms in Radiobiology," 2 Vols., New York, Academic Press, 1960.
5. Hollaender, A., ed., "Radiation Biology," 3 Vols., New York, McGraw-Hill, 1954.
6. Lea, D. E., "Actions of Radiations on Living Cells," 2nd ed., New York, Macmillan, 1955.
7. Schubert, J., and Lapp, R. E., "Radiation: What It Is and How it Affects You," New York, Viking, 1957.

Specific

1. Zirkle, R. E., in *Radiation Biology*, ed. by Hollaender, A., 3 vols., New York, McGraw-Hill, 1954.
2. Alper, T., *Radiation Research*, **5**, 573 (1956).
3. Alexander, P., in "Radiation Protection and Recovery," ed. by Hollaender, A., New York, Pergamon Press, 1960.

2. Physiological Effects of Total Body Irradiation

There are a large number of effects produced in biological systems by ionizing radiation, the modification of almost any of which could be used as a criterion of protective action. The most convenient one to use in mammals is survival, since death of the animal provides an unmistakable end point. Thus a brief discussion of the radiation-induced physiological and anatomical changes associated with the lethal process may be valuable.

For comparison of the lethal effects of different qualities of photon irradiation in different species of animals, the 30-day LD_{50} is commonly used. This term designates the dose that will kill 50 per cent of the animals within 30 days after exposure. Although the LD_{50} varies considerably depending upon the wave length of the radiation and the rate at which it is delivered, for 200 kv X-rays given at rates above 40 r/minute the LD_{50}'s (measured in air) are rather close for most adult mammalian species, ranging from about 200 r for some strains of guinea pigs to about 800 r for rabbits. On the basis of limited evidence, the 30-day LD_{50} for man has been estimated to be 400 to 500 r.

In addition to the physical factors of wave length and dose rate mentioned previously, there are physiological factors influencing the LD_{50} of X-radiation. In different strains of mice, the LD_{50}'s may vary from 400 to 700 r. Young animals (weanlings) are almost twice as susceptible as young adults, and old animals are also somewhat more susceptible. Generally, females are more sensitive than males, although the effect is small. Diet also has a slight effect on radiosensitivity; some of the results obtained are contradicting, and the influence of