Repairable Lesions in Microorganisms

Edited by

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and

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National Research Council of Canada
Ottawa, Ontario, Canada

ACADEMIC PRESS
(Harcourt Brace Jovanovich, Publishers)
London Orlando San Diego New York
Toronto Montreal Sydney Tokyo

ACADEMIC PRESS INC. (LONDON) LTD.
24-28 Oval Road,
London NW1 7DX

United States Edition published by
ACADEMIC PRESS, INC.
Orlando, Florida 32887

British Library Cataloguing in Publication Data

Repairable lesions in microorganisms.
1. Micro-organisms–Physiology
2. Deoxyribonucleic acid repair
I Hurst, A II Nasim, A
576 QP84

Library of Congress Cataloging in Publication Data
Main entry under title

Repairable lesions in microorganisms

Includes bibliographical references and index
1 Micro-organisms–Physiology. 2. Deoxyribonucleic acid repair 3 Adaptation (Physiology) I Hurst, A (Andre), DATE II Nasim, A (Anwar), DATE
QR97 A1R46 1984 576'11 84-9388
ISBN 0–12–362690–0 (alk paper)

PRINTED IN THE UNITED STATES OF AMERICA

84 85 86 87 9 8 7 6 5 4 3 2 1

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Preface

An underlying unity between all living creatures is a general biological principle illustrated by the saying that 'What is true for *Escherichia coli* is true for the elephant'. For example, some vitamins first discovered in microbes were later found in higher organisms, and recently information concerning DNA first discovered in *E. coli* has been demonstrated in man. The idea that microbes, like higher organisms, can become injured is of still more recent origin.

It has been known for at least 40 years that radiation can kill microbes, and practical applications for sterilization and food processing have been sought since these original observations were made. Non-lethal doses can injure microbes, which we now find are endowed with sophisticated repair mechanisms. The late Z. J. Ordal of the University of Illinois was one of the pioneers who 25 years ago observed that non-lethal heat can injure microorganisms. Still more recently, it has become clear that almost any physical and chemical stress first injures microbes and only then may kill them.

It is evident that conditions which injure but do not kill involve relatively low doses. At present there is no definite proof that these mild conditions are the same as those that lead to death. Large, killing doses cause a plethora of damaged sites so rapidly that it is impossible to deduce which site was first affected. This is an important concept, suggesting that the elucidation of the primary site of damage by an agent is best attempted via injury and repair studies, not by merely observing the overall condition of a fatally injured microbe.

Microbes, like other living creatures, inhabit an environment containing a multiplicity of stresses which favour mutations and evolution. One can argue that without the pressure of some form of stress, evolutionary progress becomes impossible. We cannot imagine an environment without some kind of radiation and extremes of temperature and pressure. Microbes have developed mechanisms to repair damage from all of these inescapable forces, and in this book we discuss each of these hazards in relation to microbes in general.

Although the most is known about *E. coli*, spores deserve a special mention in this preface. In spite of being so resistant to all forms of physical damage, they can nevertheless be injured, and they can repair their injury. (The possibility that life on earth started from extra-terrestrial spores cannot be overlooked.) Bacterial spores are produced by healthy organisms facing starvation; spores then remain dormant as long as conditions remain unfavourable. Germination is triggered by conditions favourable for vegetative growth, but a fraction of spores remain dormant. This fraction, often called superdormant, may be produced by microbes in response to injury in order that some individuals may persist in resistant form until conditions become safe for vegetative growth.

This book falls naturally into two parts. The first five chapters deal with damage to and repair of DNA following injury by different forms of radiation. Subsequent chapters, while not disregarding DNA damage, discuss damage and repair to other cellular sites following injury by body defenses, osmotic activity, freezing, pressure and heat.

This book is aimed at final-year students and post-graduates. However, we hope that our coverage is sufficiently comprehensive to be useful to anyone engaged in microbiological research and teaching.

August 1984

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CHAPTER 1

Introduction: Environmentally Induced DNA Lesions and Their Biological Consequences*

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I. Introduction

All organisms, from simple unicellular microbes to complex multisystem mammals, sustain injury, often of a potentially debilitating nature, from the unavoidable wear and tear of normal living. Structural damage to deoxyribonucleic acid (DNA)—the carrier of the vital genetic information required for normal cellular metabolism and reproduction—is at once the most fundamental and

*Supported in part by U.S. National Cancer Institute Contract NO1-CP-21029 (Basic).

ISBN 0-12-362690-0

the most harmful of such injuries. While much of this damage is thought to arise spontaneously, the majority of the particularly deleterious lesions is inflicted on living creatures by numerous physical and chemical agents in our environment. Examples of common biospheric hazards include: ultraviolet (UV) rays of the sun, γ-rays emitted from the nuclei of some radioactive atoms, and chemicals, both naturally occurring (e.g. cycasin from cycad plants) and man-made (e.g. nitrofurantoin, a broad-spectrum antibiotic).

Although alterations in other components, such as the plasma membrane and endoplasmic reticulum, can undoubtedly compromise the well-being of a cell, the critical site of attack by most of the well-defined environmental toxicants is the DNA. In fact, at sub-lethal doses of a given environmental agent, only with DNA is the effect sufficient to account for the cellular changes observed subsequently. RNA molecules, usually present in many copies anyway, are not appreciably inactivated at these biologically meaningful doses, nor are the activities of enzymes inhibited to any significant extent.

Since DNA constitutes life's primary information-carrying macromolecule (as evidenced by the order of biosynthesis in cells: DNA→RNA→protein), it is not surprising that living organisms should possess a battery of enzymatic DNA repair mechanisms designed to permit cellular recovery from the multitude of potentially injurious changes to which the genetic material is routinely subjected. In fact, maintenance of the integrity of the "master tape" is evidently of high priority in all life forms irrespective of their phylogenetic position, as is borne out by the observation that DNA repair systems have been found wherever they have been sought throughout the animal and plant kingdoms.

This chapter is meant to serve as an introduction to the more specialized topics to be addressed later. To this end, we shall undertake to describe (in terms readily understood by non-specialists) the basic principles and prefatory knowledge deemed essential to fostering an appreciation of the main subject of this treatise—that is, the cause, nature and effect of repairable lesions in microorganisms. Five major topics will be considered in turn. First, the diversity of the basic modes of action of representative toxicants, both those encountered in the natural habitat and those developed by man, will be illustrated; attention will be focussed on those agents that are deleterious primarily as a result of their ability to attack DNA because such so-called "genotoxic agents" are believed to represent a formidable challenge to the health and prosperity of living things. Second, a description will be given of the major classes of lesions which are introduced into DNA by these agents. Third, we will detail the metabolic responses of simple biological systems to such damage, concentrating on the various enzymatic mechanisms that have evolved in different bacteria and in their viruses to assure the preservation of their genetic heritage. Fourth, consideration will then be given to the dire consequences to the microorganism when too many defects are permitted to remain in its DNA. And fifth, we will conclude with a brief discussion of how this knowledge about environmentally induced DNA damage and its imperfect enzymatic repair in microbes has played a key role in advancing our understanding of the cause and course of human disease in general and of cancer—one of the major scourges of modern man—in particular. As the reader shall see, immense practical and academic benefits have been (and will surely continue to be) derived from the study of repairable lesions in microorganisms.

II. Repairable Lesions in DNA

A. CAUSATIVE AGENTS AND THEIR MODES OF ACTION

With few exceptions (e.g. viruses, such as λ bacteriophage), the multitude of genotoxic agents present in the environment can be divided into two major classes: radiations and chemicals.

1. *Radiations*

All life forms are continuously exposed to different types of electromagnetic radiation, ranging from high-energy cosmic and X-rays to low-energy radio and radar waves. Each type is considered to involve energy transfer through space or matter by means of oscillating electric and magnetic fields. According to modern physical theory, electromagnetic radiation consists of discrete packets of light quanta or photons which have properties of both waves and particles; as a wave, a photon can be characterized by its wavelength, and as a particle, it can be described by its quantum of energy. The energy of a photon is inversely related to wavelength by the equation:

$$E = hc/\lambda$$

where E is the energy of a single photon in joules, h is Planck's constant (6.626×10^{-34} J sec), c is the velocity of light (2.997×10^{8} m sec^{-1}) and λ is the wavelength of the particular type of radiation in meters. Ultraviolet radiation, having shorter wavelength than visible light, is more energetic than visible light, whereas infrared radiation, having longer wavelength than visible light, is less energetic. The different types of radiation in the electromagnetic wave spectrum are shown in Fig. 1.

There exists a second major class of radiation besides that transmitted in the form of electromagnetic waves. This other class, known as particulate radiation, is comprised of rapidly moving, highly energetic atomic particles, such as electrons and protons. These particles are emitted from natural radioactive materials and from man-made accelerating machines (e.g. cyclotrons) and nuclear reactors. The most common types of particulate radiation, their physical properties and their sources are summarized in Table I.

The biological effects of electromagnetic radiation can be either beneficial or

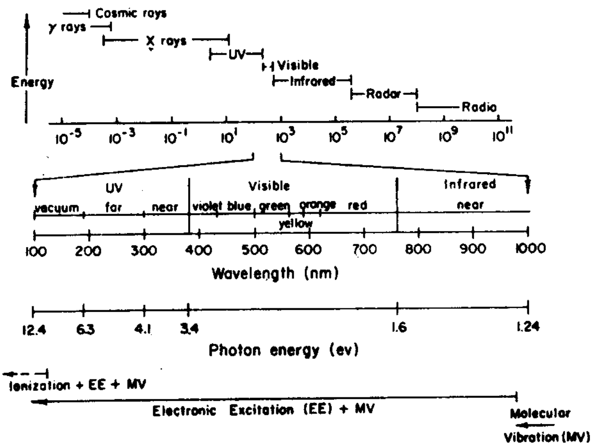

Fig. 1. Wavelength of electromagnetic radiation on a logarithmic scale and of three types of radiation—UV, visible and infrared—on an expanded linear scale. Above these scales are indicated the various types of radiation throughout the entire electromagnetic spectrum; the upper and lower wavelength cutoffs of each type are not sharp, as portrayed here. Below the scales are given the corresponding photon energies and the primary chemical events that can occur in the indicated portions of the spectrum. Note that any given photon can also cause events associated with photons of lower energy.

deleterious, depending upon the energy and the amount of the particular type of radiation to which an organism is exposed. Obviously, visible light is of key importance to the very survival and evolution of many life forms, permitting such essential chemical reactions as photosynthesis in plants (thus producing carbohydrates and proteins, the basic organic sources of food and energy for animals) and vision in animals. Low levels of UV light appear essential to certain species, notably *Homo sapiens* wherein its action is required for the formation of

Table I. *Types of Particulate Radiation*

Particle type	Charge[a]	Mass[b]	Atomic equivalent	Source
β-Particle	−1	1/1843	Electron (ejected from radioactive nuclei)	Accelerating machines, radioactive materials
Neutron	0	1	Neutron (produced by nuclear fission)	Nuclear reactors, accelerating machines
Proton	1	1	Nucleus of hydrogen atom	Accelerating machines
α-Particle	2	4	Nucleus of helium	Radioactive materials, accelerating machines

[a]Relative to that of an electron, defined as the unit negative charge.
[b]Relative to the mass of the hydrogen nucleus (1.672×10^{-27} kg), defined as the unit mass.

vitamin D. On the other hand, excessive amounts of solar UV and of X-rays or other forms of highly energetic radiation are extremely detrimental to living systems, leading to genetic mutations and lethal events in individual cells, and cancer and other life-threatening conditions in higher organisms.

Unlike the arbitrary divisions shown in Fig. 1, the actual boundaries between the different forms of hazardous radiation in the short wavelength end of the electromagnetic spectrum are not sharp. Nevertheless, for convenience, 100 nm is commonly taken as the lower cut-off wavelength for UV, and hence serves to arbitrarily separate non-ionizing radiation (i.e. UV, visible and other kinds of long-wavelength radiation) from ionizing radiation (i.e. X, γ and cosmic rays, plus high-energy particulate radiation), and accordingly the sciences of photobiology and radiobiology, respectively.

a. Non-Ionizing Types. When considering detrimental effects on living systems, essentially the only relevant type of non-ionizing electromagnetic radiation is UV light. In Fig. 1, the UV region is divided into vacuum UV (100–190 nm), far-UV (190–300 nm) and near-UV (300–380 nm). Of the UV wavelengths

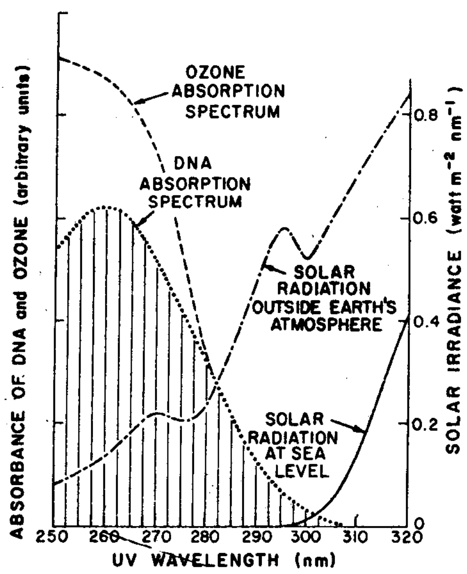

Fig. 2. Absorption spectra of DNA and ozone. For comparative purposes, the two spectra have been normalized to the same absorbance at 290 nm. The solar irradiation spectra outside the earth's atmosphere and at sea level are also shown. Note that because of absorption by stratospheric ozone, only a small fraction of solar mid-UV reaches the earth's surface and that the solar UV wavelengths which overlap with the absorption spectrum of DNA have been drastically reduced. [From Gentner and Myers (1980) with permission of Atomic Energy of Canada Limited.]

impinging on the earth, air and water effectively absorb all vacuum UV rays, while gaseous components of the upper atmosphere, especially stratospheric ozone, selectively filter most of the far-UV (<290 nm) (see Fig. 2); indeed, the recent well-publicized concern over the increased release into the stratosphere of man-made chemicals which can decompose ozone, including freons (in spray can propellants and refrigerants) and nitrogen oxides (from supersonic jets), emphasizes the immense importance of the ozone layer in protecting terrestrial life from severe DNA damage (likely culminating in death) by far-UV rays. Photobiologists further define mid-UV as the radiation between 290 and 320 nm, in recognition of the fact that this region is responsible for the lion's share of the harmful biological effects of sunlight; in fact, doses of near-UV (320–380 nm) must be 100 to 1000 times greater than that of mid-UV to produce equivalent biological damage. Fortunately, at the earth's surface, the intensity of mid-UV rays is exceedingly low compared to near-UV and visible rays (Fig. 2).

Let us now discuss the mode of action of non-ionizing radiation on living matter. Of course, a photon must be absorbed by one or more components of the exposed organism in order for the radiation to produce a biological effect, since it is the absorbed energy that performs the work needed to induce a chemical change. The chemical alterations that can occur upon transfer of energy from radiation to biological materials are dependent in large part upon the amount of energy characteristically imparted by a quantum of a given radiation type. Infrared radiation yields quantum energies of approximately 0.1–1.6 electron volts (ev) (Fig. 1), where 1 ev equals 1.602×10^{-19} J. Absorption of this quantity of energy is sufficient only for inducing molecular vibration (bond stretching and bending resulting in displacement of atomic nuclei) in the target molecules. Hence the sole consequence might simply be an increase in biochemical reaction rates due to dissipation of this radiant energy as heat. The energies (~2–6 ev) delivered by quanta of UV (and, to a lesser extent, of visible light) can produce, in addition to molecular vibration, electronic excitation—that is, elevation of an outer orbital or valence electron to a higher energy level, thereby converting the molecule from a ground to an excited state. A molecule in such a state has a greater probability of undergoing chemical reaction than when in the ground state.

It is important to realize that absorption of non-ionizing radiation by a cellular component is a precise phenomenon. Whether a given type of radiation can affect a molecule depends on whether the quantum delivered can raise the molecule to a higher vibrational or electronic excited state. That is, the reason why only certain wavelengths of radiation are absorbed by a particular molecule is that the energy of the absorbed quantum must exactly equal the excitation energy for some permitted excited state of the molecule. In a cell, nucleic acids and proteins are the main constituents responsible for the absorption of UV light. In the region 240–290 nm, the nucleic acids are particularly important, absorbing 10- to 20-fold as much energy as equal weights of proteins. This is because the principal absorbers of far-UV are molecules containing conjugated bonds (alternating single and double bonds) and, in the nucleic acids, all four constitutive bases are aromatic and hence display high absorption. (Likewise, the primary molecular components of proteins that absorb UV are the aromatic amino acids, tryptophan and tyrosine, followed by phenylalanine, cysteine and cystine.)

A biomolecule in an excited state can return to the original ground state by emitting the excitation energy in the form of light (i.e. fluorescence or phosphorescence) or heat, or the excited molecule can undergo a chemical transformation to form a new species. In DNA, the latter predominates. Namely, a base in an excited state tends to interact electronically with an adjoining ground-state base to form an excited-state dimer, or exciplex. The energy level of the exciplex is lower than that of the initially excited monomer, and the excess energy is released in the form of fluorescence. The exciplex is thought to play a key role in DNA photochemistry, as it is probably the excited-state precursor species for the formation of cyclobutyl pyrimidine dimers (Section II,B).

It is this highly selective absorption of far-UV by the genetic material (absorbance maximum ~260 nm) which has made the low-pressure mercury lamp (emitting ~85% of its radiant intensity at 254 nm) such a potent germicidal source and also a useful laboratory tool for the study of DNA damage and its repair. This relatively simple and specific interaction between far-UV and DNA contrasts sharply with the complexities arising from the non-selective disposition of energy imparted by ionizing radiation, as will presently be discussed.

b. Ionizing Types. While the high energies ($\geqslant$12 ev) transmitted by electromagnetic radiation of wavelength 100 nm and shorter (e.g. X- and γ-rays) are also absorbed primarily by valence electrons, the photon energy transferred greatly exceeds the electron binding energies of biomolecules. This is sufficient to produce not only molecular vibration and electronic excitation, but also, more importantly, ionization—that is, the acquisition of a charge by removal of an electron (or electrons), yielding a highly reactive, electrically charged species, "ion", and one or more free electrons. The effect of highly energetic particulate radiation is ultimately the same as that of ionizing electromagnetic radiation. Different types of particulate radiation transfer their high energy by collision with the orbital electrons or atomic nuclei of the material through which they traverse (Table I). Charged particles, such as α-particles and protons, transfer energy to the valence electrons and, by so doing, leave the molecule or atom in an ionized state. Neutrons, being unchanged, are able to pass through electron orbits and impinge upon the nucleus of a target atom, usually hydrogen, which then recoils as a fast moving hydrogen ion (i.e. proton). This capacity of high-energy electromagnetic and particulate radiations to produce ionizations in target molecules has led to their collective designation as ionizing radiation, thus distinguishing them from UV and other low-energy forms of non-ionizing electromagnetic radiation.

On passing through biological material, ionizing radiation does not transfer its energy uniformly but instead deposits it in discrete packages well separated in space. As a result, relatively enormous amounts of energy are absorbed by a few constituents of some cells while other cells receive little, if any. In fact, ionizations rarely arise singly but occur in multiple events referred to as "clusters" or "spurs". As a case in point, X-rays produce so-called secondary electrons which can in turn ionize the neighboring material in their pathway during the course of giving up their kinetic energy. Indeed, many such subsequent ionization events usually occur for each primary ionization, and hence most of the injury induced by X-rays is due to the secondary electrons, and not to the initial ionization event. This uneven distribution of energy goes some distance towards explaining why the absorption of a quantity of radiant energy so small as to go virtually unnoticed by the exposed organism can eventually lead to serious illness; for example, the amount of energy imparted to a person by a lethal dose of X-rays, totalling ~4–5 grays (Gy), is comparable to the heat energy absorbed upon drinking a hot cup of coffee or sunbathing for only minutes on a hot day. (Note: 1 Gy is defined as the absorption of 1 J of radiant energy per kilogram of material.)

Although high-energy radiations, both electromagnetic and particulate, interact similarly with water or organic materials in living matter to produce ionizations, identical quantities of energy deposited (i.e. the same number of ionizations produced) by different types of radiation can differ in their effectiveness in inflicting biologically meaningful damage. A key factor in the so-called relative biological effectiveness (RBE) of a particular type of radiation (compared to a reference type, i.e. 250 kvp X-rays) is the spatial distribution of the ionization events along its track. X- and γ-rays, as well as fast-moving electrons (β-rays), produce ionization events that are infrequently spaced along comparatively long paths; they are thus known as radiation of low linear energy transfer (LET, defined as mean energy released in kiloelectron volts per micrometer of biological material traversed). Protons (including those produced by neutrons through collisions) and α-particles have a higher probability of interaction with atoms and therefore a very short range; thus these charged particles cause ionizations densely spaced along short tracks and therefore constitute examples of high LET radiation. Consequently, the ionization events will be distributed more diffusely over a larger volume by low compared to high LET radiation. In general, high LET radiation is more damaging per unit of dose (i.e. exhibits a higher RBE) than low LET radiation presumably because of the requirement to produce a number of ionizations within a certain small volume in order, for example, to inactivate the critical cellular target, which is almost certainly DNA.

Ionization in biological systems, irrespective of its complexity, occurs largely within individual cells. Two main types of intracellular action can be distinguished: (1) direct action, in which the radiant energy is absorbed directly by the critical target molecule; and (2) indirect action, in which the energy is first