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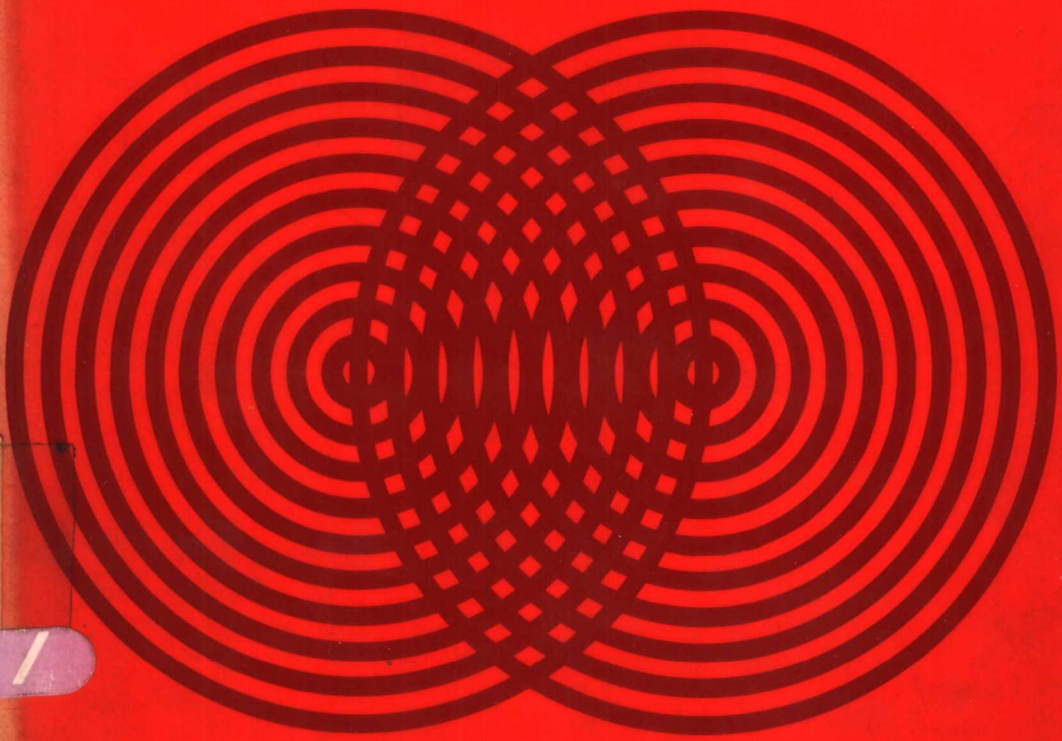
Walter Harm



Biological effects of ultraviolet radiation

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FOREWORD

The origins of this series were a number of discussions in the Education Committee and in the Council of the International Union of Pure and Applied Biophysics (IUPAB). The subject of the discussions was the writing of a textbook in biophysics; the driving force behind the talks was Professor Aharon Katchalsky, first while he was president of the Union, and later as the honorary vice-president.

As discussions progressed, the concept of a unified text was gradually replaced by that of a series of short inexpensive volumes, each devoted to a single topic. It was felt that this format would be more flexible and more suitable in light of the rapid advances in many areas of biophysics at present. Instructors can use the volumes in various combinations according to the needs of their courses; new volumes can be issued as new fields become important and as current texts become obsolete.

The International Union of Pure and Applied Biophysics was motivated to participate in the publication of such a series for two reasons. First, the Union is in a position to give advice on the need for texts in various areas. Second, and even more important, it can help in the search for authors who have both the specific scientific background and the breadth of vision needed to organize the knowledge in their fields in a useful and lasting way.

The texts are designed for students in the last years of the standard university curriculum and for Ph.D. and M.D. candidates taking advanced courses. They should also provide a suitable introduction for someone about to begin research in a particular field of biophysics. The Union is pleased to collaborate with the Cambridge University Press in making these texts available to students and scientists throughout the world.

Franklin Hutchinson, Yale University
Watson Fuller, University of Keele
Lorin Mullins, University of Maryland
Editors

PREFACE

The killing of cells by ultraviolet (or UV) radiation, in particular by wavelengths present in sunlight, has been studied for more than a century. The ultraviolet's mutagenic action was experimentally established not long after discovery of the mutagenicity of ionizing radiations by H. J. Muller in 1927. Concomitantly, UV action spectra for the killing of cells and viruses and for mutagenesis indicated that these effects were due to energy absorption in nucleic acids, substances then known to be part of the chromosomes, and as it turned out later, those actually carrying the hereditary information. Aside from such early roots, however, UV photobiology is a relatively recent area of research, comprising in essence the achievements of the past 20 to 30 years. Its development during this period paralleled the rapid progress in genetics and molecular biology, resulting mainly from the experimental use of microorganisms and viruses.

Basically, research in UV photobiology has employed the radiation as a tool to damage organisms in a fairly specific manner, at least more specific than ionizing radiations do. Investigating the consequences of such damage not only permitted conclusions regarding the UV-absorbing material and the ways in which the damage interferes with vital cellular processes, but also led to the observation of recovery effects. Work by A. Hollaender in the middle 1930s gave the first evidence for them, but their general significance was not recognized until the later forties and early fifties. The study of recovery phenomena indicated that, in contrast to earlier concepts, the eventual fate of an irradiated organism is highly conditional, rather than being fully determined at the time of energy absorption. Not only did these achievements offer explanations for otherwise irreconcilable discrepancies between the results obtained in different laboratories, subsequent studies on recovery phenomena also revealed their molecular basis: the existence of a variety of sophisticated and highly efficient repair processes enabling the cells to cope with otherwise unbearable conditions. They are of great importance in protecting organisms against radiation damage from our natural UV source, the sun, as well as against damage from a wide variety of other agents. Moreover, it is now evident that some of the reaction steps involved in repair play at the same time a significant role in general cellular maintenance processes and such basic phenomena as genetic recombination and DNA replication. The use of UV radiation has been basically involved in these important discoveries.

When Franklin Hutchinson, the chairman of the Editorial Board of this series of biophysics textbooks, approached me with regard to writing a book on *Biological Effects of Ultraviolet Radiation*, I accepted with mixed feelings. On the one hand, I was grateful for the opportunity to summarize the results, as well as my own views and thoughts, in an area of research that I have been associated with for the past 25 years, and in which my writing had so far been only in the form of original papers and review articles. On the other hand, it was obvious that the task of covering a field in which interpretations and concepts are still in a considerable flux, and to which hundreds of scientists contribute the results of their research almost daily, is a difficult one. My final decision was facilitated by the editor's request that the textbook be short, comprising essentially what he felt I carry around in my head. Although the latter turned out to be too optimistic in many instances, I was aware that the more time-consuming parts of the job would be: (1) the decision as to what one can consider of sufficient importance to provide graduate and advanced undergraduate students, or scientists established in other areas of research, with a useful background for entering this field; (2) the presentation of the material in a form easy to comprehend, particularly with regard to those potential readers unfamiliar with the inherent genetic and quantitative approaches. My apologies, if I have not always succeeded in these respects.

Among the rewards for writing a textbook is the author's satisfaction in presenting the facts, concepts, and his own thoughts in his field of competence in a didactically most desirable form. Full justification of the effort, however, requires the existence of a definitive need for such a book. The implicit criterion that the book must differ significantly from others on similar topics is not difficult to meet in the present case. There exist several volumes with contributions from research symposia and excellent review articles on various aspects of UV photobiology, published in journals, in periodicals, or in the form of multiauthored books. They are invaluable as a source of information for the advanced scientist but necessarily inadequate as introductory texts. A recently published work, *The Science of Photobiology*, edited by K. C. Smith (Plenum, 1977) covers the whole discipline of photobiology, including photosynthesis, photomorphogenesis, bioluminescence, and many other fields, and contains excellent contributions from many authors in their specific areas of competence. But only the chapter "Ultraviolet Radiation Effects on Molecules and Cells" coincides to a major extent with the contents of this book. Among textbooks, *Introduction to Research in Ultraviolet Photobiology* by J. Jagger (Prentice-Hall, 1967) puts its main emphasis on the techniques involved in UV-photobiological research. *Molecular Photobiology* by K. C. Smith and P. C. Hanawalt (Academic Press, 1969) covers a similar area, as does this book. However, almost 10 years have passed since publication of *Molecular Photobiology*, and in their contents the

two books complement one another in several respects. Unquestionably, the emphasis on biological problems in the present book reflects the author's own research preference and his original training as a biologist.

The first three chapters of the book are designed to provide the reader with the background in chemistry and physics essential to an understanding of the biological effects, to which all of the remaining 10 chapters are devoted. The reader may feel that Chapters 7 and 8, dealing with recovery, repair processes, and related phenomena observed after UV damage, are treated in more detail than seems appropriate in comparison with other chapters. Perhaps this amounts to overemphasis of my own research area. But in all fairness, one can probably say that during the past 10 to 20 years hardly any other branch of UV photobiology contributed more to our general biological knowledge than the study of repair and recovery.

It is my pleasure to acknowledge the help of many colleagues, and the publishers of their work, for the kind permission to use their graphs or other illustrations in this book. The names are too numerous to mention them all here; acknowledgments are given at the appropriate places. Particular thanks are owed Dr. Franklin Hutchinson for the encouragement to my writing, and my colleague Dr. Claud S. Rupert, with whom I have had for the past 15 years close scientific relations in several areas covered by the book. These resulted in a number of joint publications as well as in an earlier attempt at writing together a comprehensive monograph on UV photobiology of microorganisms. We abandoned this effort, simply because the literature was turning out results faster than we were able to digest them for the purpose of writing, taking into account all other commitments and problems at a newly established university campus. Nevertheless, this apparently futile exercise was of considerable help in the preparation and writing of the present text, notably the first three chapters, which cannot deny the physicist's influence.

My thanks also include Dr. Michael H. Patrick for critically reviewing several chapters of the book, and Mr. H. Thomas Steely, Jr., a graduate student in the Molecular Biology Program at The University of Texas at Dallas for reading the manuscript and giving me his views as a potential user of the textbook. I am particularly indebted to Sally Rahn for secretarial help and for the typing of the manuscript.

Walter Harm

August 1979

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1 Introduction to ultraviolet radiation

1.1 General characteristics

Ultraviolet radiation or ultraviolet light (UV) is part of the spectrum of electromagnetic waves, covering the *interval between X-rays and visible light.* Although real boundaries between these different kinds of radiation do not exist in physical terms, they are dictated by practical considerations. Perception by the human eye begins at about 380 nm,[1] which therefore constitutes the upper wavelength limit of the UV spectrum. Specifying a lower wavelength limit is far more arbitrary, but a reasonable figure is 100 nm, below which radiations ionize virtually all molecules. For the study of biological effects of UV radiation there is a practical lower limit at about 190 nm. Shorter wavelengths are strongly absorbed by water and air, making it mandatory to irradiate in vacuum (vacuum ultraviolet). Not only does this require special equipment, it is also incompatible with experimentation on most living systems. Therefore, with few exceptions, we consider for the biological effects of UV radiation essentially the wavelength range from 190 to 380 nm.

Electromagnetic radiations transfer their energy in units of *energy quanta*, or *photons.* As first stated by Planck,

$$E = h\nu \tag{1.1}$$

that is, the energy of a photon (E) is directly proportional to its frequency of vibrations per second (ν), with h being Planck's constant (6.62×10^{-27} erg · sec or 6.62×10^{-34} Joule · sec). Because $\nu = v/\lambda$ (where v is the velocity of light, and λ is the wavelength), equation (1.1) can also be written in the form

$$E = hv/\lambda \tag{1.2}$$

E and ν of a photon are independent of the medium transmitting the radiation, whereas v, and consequently λ, varies. It is nevertheless customary to characterize UV radiation in terms of its wavelength, namely, by specifying the wavelength that the radiation would have in vacuum, where $v = 2.99 \times 10^{10}$ cm/sec.

For expressing the energy of a single UV photon, the erg and the Joule (J) are rather large units. Conventionally one uses the electronvolt (eV), defined as the energy gained by an electron in passing through a potential difference of 1 volt, which equals 1.6×10^{-12} erg or 1.6×10^{-19} J. Ac-

cording to equation (1.2), 1 eV corresponds to a photon of 1.24×10^{-4} cm (or 1240 nm) wavelength, and it follows from the inverse proportionality of the energy of a photon with its wavelength that

$$E[\text{eV}] = \frac{1240}{\lambda[\text{nm}]} \tag{1.3}$$

For photochemical purposes it is sometimes useful to express photon energies in kilocalories per einstein because absorption of 1 einstein ($= 6.02 \times 10^{23}$ photons) can excite 1 mole of the absorbing substance. In these terms, the photon energy (E), and thus the excitation energy of a molecule (E_{exc}), are related to the wavelength by

$$E[\text{kcal/einstein}] = E_{exc}[\text{kcal/mole}] = \frac{28{,}590}{\lambda[\text{nm}]} \tag{1.4}$$

From equations (1.3) and (1.4) it follows that the energies of photons within the 190 to 380 nm wavelength range vary by at most a factor of 2, namely, from 6.5 to 3.3 eV, or from 150 to 75 kcal/einstein.

Radiation of the ultraviolet and the adjacent visible spectral range (as well as all other less energetic radiation) is summarily called *nonionizing radiation*, as opposed to *ionizing radiation*. The latter is represented in the electromagnetic spectrum essentially by X-rays and gamma-rays; other kinds of ionizing radiations (such as beta-rays, alpha-rays, protons, etc.) consist of ionizing particles.

The main reason for this distinction is their interaction with matter: Ionizing radiations, in contrast to nonionizing radiations, are capable of ionizing all kinds of atoms and molecules. Absorption of nonionizing radiations typically leads to *electronic excitation* of atoms and molecules (see Section 1.2); however, ionization already begins in the ultraviolet spectral region around 200 nm and, depending on the type of atoms or molecules, becomes more relevant as the wavelengths further decrease. Most organic molecules require wavelengths below 150–180 nm in order to dissociate an electron (and thus to leave behind a positive ion). In view of the wavelengths used in most biological UV experiments and the molecules primarily affected by the energy absorption, we can for all practical purposes, exclude ionizations as one of the possible immediate consequences of UV-irradiation in biological materials.

1.2 Electronic excitation

An atom or molecule absorbing a UV photon assumes for a period of 10^{-10} to 10^{-8} sec an *excited state*, in which the energy of the electrons is increased by the amount of photon energy. Because the number of possible energy states for the electrons of an atom or molecule is finite, only photons of

specific energies (i.e., specific wavelengths) can be absorbed by an isolated atomic or molecular species. Consequently, UV absorption spectra of gas atoms at low pressure usually consist of sharply defined, discrete *absorption lines.* In simple molecules, whose rotational and vibrational energy states can also be affected by the absorbed photon, the spectral lines occur in closely spaced groups, or *absorption bands.* The larger and the more complex the molecule, the more closely spaced become the line patterns of the bands. In the solid or liquid state, where interactions with neighboring molecules prevent free rotation and disturb the energy levels, the fine details disappear from the observed absorption spectrum, and the bands become a *continuum* of smoothly changing intensity with the wavelength. This is characteristic of the UV-absorption spectra of the most important biomolecules, such as nucleic acids and proteins (see Figure 3.2).

The excitation energy provided by UV photons is much higher than the energy of thermal motions of the molecules at physiological temperatures. The latter is of the order of Boltzmann's constant times the absolute temperature, which, at 27°C, amounts to only 0.026 eV/molecule (= 0.60 kcal/mole), in contrast to the 3.3 to 6.5 eV/molecule (or 75 to 150 kcal/mole) available from UV absorption. Consequently, the absorbing molecules temporarily assume energy levels that otherwise they would never attain and thus acquire properties differing considerably from those effective in ordinary chemistry.

The lifetime of a molecule in its usual excited state (10^{-10} to 10^{-8} sec), which is still long compared with the time required for the energy absorption itself (approximately 10^{-15} sec), can be greatly extended if the excited electron is trapped in an (energetically somewhat lower) *triplet* excited state. In contrast to the usual *singlet* state, the triplet state is characterized by two electrons with *unpaired spin.* Because the return from the triplet state to the ground state is "forbidden" (i.e., occurs at a low probability), the triplet may last 10^{-3} sec or even longer and is, therefore, called *metastable.*

As an excited electron returns to a lower energetic state, its excess energy may be disposed of in several ways:

1. It can be emitted as a photon, resulting in *fluorescence.* Fluorescent light is recognized by its usually longer wavelength, compared with the exciting radiation. Emission from molecules in the metastable excited state occurs over a longer period of time and is called *phosphorescence.*
2. The excitation energy can be *dissipated as thermal energy* in the course of collisions with other molecules.
3. The energy may cause the excited molecule to undergo a *photochemical reaction* that otherwise would not occur. The likelihood for this to happen increases with the lifetime of the excited state and is thus greatly enhanced for the triplet state. Photochemical reactions are the immediate effects of UV radiation in biologically relevant molecules, and constitute the basis for the observed photobiological phenomena. They will be discussed in more detail in Chapter 3.

1.3 Biological effectiveness

Although the photon energies within the biologically applicable UV spectrum vary by no more than a factor of 2, equal numbers of incident photons can cause photochemical (and consequently photobiological) reactions differing in quantity by several orders of magnitude. This rather general observation indicates wide variations in the absorption of photons at different wavelengths by the relevant biomolecules. Obviously only absorbed, but not transmitted or reflected, radiation energy can be photochemically effective (Draper-Grotthus principle). We will see later that the majority of biological UV effects are due to photochemical reactions in nucleic acids, which constitute the genetic material of all cellular organisms and viruses. Protein effects generally play a minor role, but are relevant in some cases.

Nucleic acids and most proteins have their absorption maxima well below 300 nm and absorb little at wavelengths above 300 nm. Therefore, it is not surprising that biological UV effects produced by radiation below 300 nm are infrequently observed at longer wavelengths. For this reason, it is customary to subdivide the UV spectrum into *near UV* (300-380 nm) and *far UV* (below 300 nm), the adjectives near and far indicating the relative distance from the visible spectral range. Because far UV is much more effective than near UV with respect to inactivation of microorganisms (which is one of the technical applications of UV radiation), it is also called *germicidal UV*.

To separate the far and near UV region just at 300 nm is convenient, but the borderline could as well be placed somewhat above or below this point. As a matter of fact, the region *surrounding* 300 nm (from approximately 290 to 315 nm) is in several respects very critical. In this region absorption of most nucleic acids and proteins diminishes rapidly, so that it becomes unmeasurable at still higher wavelengths. Conversely, the solar emission spectrum reaching the earth's surface contains mainly near UV (besides visible and infrared light); the shortest wavelengths are usually somewhere between 290 and 315 nm, depending on many factors (see Section 11.1). Furthermore, the short wavelength cutoff in the transmission of some of the commercial glasses falls into this region.

If, for simplicity, one wants to identify measurable nucleic acid absorption with the far-UV range, and to identify the solar emission spectrum with the near-UV range, one would have to say that the two ranges overlap in the small region from approximately 290 to 315 nm. Not only does this avoid semantic confusion, but it also emphasizes the fact that effects characteristic of both far and near UV irradiation may occur in this region at comparable rates. This overlap region is indeed of considerable theoretical and practical significance. The medical literature, primarily concerned with the dermatological effects of UV radiation, often distinguishes three UV spectral regions: UV-A (>315 nm), UV-B (280-315 nm), and UV-C (<280 nm), with most interaction between sunlight and the human skin occurring in the UV-B re-

gion. Although such a subdivision is certainly useful, it has not become popular in the biophysical literature.

1.4 Sources of ultraviolet radiation

For reasons pointed out previously, most biological experiments require a UV source emitting appreciably in the far UV. Thus the lamp envelopes and any optical components of the irradiation equipment must consist of *quartz* or material of similar transmittance because ordinary glasses are opaque for far-UV wavelengths. The following paragraphs will briefly summarize some technical facts that are fundamental for understanding the text and for carrying out simple experimental work in UV photobiology. For more detailed information in this regard the reader is referred to the textbook by Jagger (1967).

1.4.1 UV sources with broad spectral emission

Early experimental studies of biological UV effects frequently employed UV sources with a broad continuous emission spectrum, for example: *hydrogen-*, *xenon-*, or *high-pressure mercury-vapor* discharge lamps. There has been some virtue in the fact that with this type of equipment one is less likely to overlook effects characteristic of only a small region of the UV spectrum. Furthermore, a broad spectral range may sometimes be favored for solving problems of an applied nature. However, regarding basic UV-photobiological research, the present state of knowledge usually requires a quantitative correlation of the observed biological effects with defined, limited spectral regions.

1.4.2 Limitation of spectral regions

A broad emission spectrum can be narrowed by inserting appropriate *optical glass filters* or *liquids*. Suitable combinations of such filters may be chosen for transmission of a rather confined wavelength region, or for the selection of a single wavelength from a line emission spectrum.

1.4.3 Monochromatic UV radiation

Commercially available *monochromators* with quartz-transmission or aluminum-reflection optics are generally used in biological experiments for isolating a single wavelength from a line emission spectrum or a narrow wavelength band from a continuum. If such an instrument is not available, *interference filters*, preferably in combination with a UV source providing a suitable line emission spectrum, can be used to achieve adequate spectral monochromasy. Such filters are now also available for far-UV wavelengths (see Appendix in Jagger, 1967).

1.4.4 Germicidal lamps

Radiation at 254 nm (or, more accurately, 253.7 nm) is obtained at high intensity from low-pressure mercury-vapor discharge lamps. Because of their commercial use for sterilizing air, water, and so on, they are widely known as *germicidal lamps.* As shown in Table 1.1, approximately 95 percent of the total UV emission of a germicidal lamp, or more than 97 percent of the far-UV emission, is at 254 nm. Because in addition this wavelength is nearly maximally effective for many UV-photobiological experiments (owing to its closeness to the absorption maximum of nucleic acids), such a lamp can be considered, for most experimental purposes, a *quasi-monochromatic UV source.* Because of the low cost and ease of handling of these lamps, they are used for the majority of published work; in fact, they are in many laboratories the only source of UV radiation.

It is important to notice that some types of germicidal lamps produce *ozone* from oxygen in the air, which is easily smelled in proximity to the lamp. Ozone production is essentially due to emission at 185 nm, a mercury line that is transmitted through a lamp envelope consisting of pure quartz. Lamp envelopes made of Vycor, or of quartz with mineral impurities, do not transmit 185 nm; therefore, these lamps are called ozone-free. Because the biological effects of 185 nm UV are expected to differ substantially, both in quantity and quality, from those of 254 nm, and because the in-

Table 1.1. *Distribution of energy emitted by a germicidal lamp*

	Percent relative emission within the region[a]		
Wavelength (nm)	248–435 nm	248–365 nm	248–313 nm
248	0.1	0.1	0.1
254	88.5	95.2	97.4
265	0.1	0.1	0.1
280/289	0.1	0.1	0.1
297	0.3	0.3	0.3
302	0.2	0.2	0.2
313	1.7	1.8	1.9
334	0.1	0.1	–
365	1.9	2.0	–
405	2.0	–	–
435	5.0	–	–

[a] The energy in a particular wavelength is expressed as the percentage of either the total emission in the 248–435 nm region, or emission in the UV region only (248–365 nm), or emission in the far UV region (248–313 nm). Figures are calculated from data obtained through the courtesy of General Electric Corp. for the lamp G 30T8.

tensity of 185 nm radiation would greatly depend on the individual lamp and the experimental conditions, it is generally recommended that ozone-free lamps be used. Nevertheless, the strong absorption of 185-nm photons in air makes it relatively safe to irradiate with an ozone-producing lamp at a distance of 25–30 cm, where the 185-nm intensity is several orders of magnitude lower than at the lamp surface.

1.4.5 Solar ultraviolet radiation

Sunlight is the only source of UV radiation to which many organisms are exposed in their natural environment. Hence the study of solar UV effects is of general biological interest and deserves attention. Although the experimental use of solar radiation encounters greater difficulties than the use of a technical UV source in the laboratory, our increasing knowledge in the field of UV photobiology makes a correct interpretation of sunlight effects feasible. A more detailed description of the biological effects of solar UV radiation will be given in Chapter 11.