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

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

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.

2 The study of biological UV effects

2.1 Biological objects

Investigations of biological UV effects have largely employed microorganisms, in particular *bacteria* and *bacterial viruses* (often called bacteriophages, or phages), and to lesser extent *yeasts*, other *fungi*, *animal viruses*, or *plant viruses*. More recently, *mammalian and other vertebrate cells* in culture have become important objects for UV studies, partly because of the awareness of UV carcinogenesis (Chapter 12) and other adverse UV reactions with the human skin (Section 11.4), and partly because of the interest in mammalian repair systems in general, which are best investigated after UV damage (Chapters 7 and 8). Investigations of UV effects in larger organisms, notably protozoa, insects, and spermatophytic plants, are widely scattered in the literature. Because such work encounters greater difficulties regarding irradiation techniques and dosimetry than the microbial work, the results and interpretations have been more open to criticism.

The choice of a biological object for UV studies depends on several things. A decisive factor, besides the scientific background and experience of the investigator, is often whether the purpose of the study is of basic-scientific or of applied-scientific character. In the latter case, use of a particular organism is sometimes dictated by the nature of the problem, irrespective of the suitability of the organism for experimental work. In contrast, UV studies carried out to obtain basic scientific insights will generally employ organisms most appropriate for the experimental approaches envisaged.

In UV photobiology, as in any other area of research, a large amount of knowledge originates from *model systems*. When a suitable organism has been extensively studied through a considerable period of time and thus many of its characteristics are well established, further work is greatly facilitated by the existence of appropriate techniques and the availability of a great variety of mutant strains. Consequently, it is most reasonable to employ the same organism, rather than any other, for answering newly arising scientific questions, unless there are compelling reasons to do otherwise. One reason to use sometimes rather different objects is to get an indication as to whether the scientific knowledge gained with the model species is of general validity. But in this brief introduction into the biological UV effects we will particularly concentrate upon those few objects that have been commonly used and from which most of our present knowledge has been derived.

The preference for bacteria, phages, and some other microorganisms in UV-photobiological studies is well justified. Their very high reproduction rate and small size, the ease and inexpensiveness of obtaining large individual numbers, make them ideal objects for quantitative work. For example, 1 ml liquid culture of the bacterium *Escherichia coli* may contain 10^9 to 10^{10} cells, which within a day could produce the same number of macroscopically visible colonies. Appropriate selection techniques after UV irradiation may permit recognition of a few colonies with altered properties, resulting from a few altered cells among the vast number plated. Another advantage of using bacteria and viruses in UV-photobiological research is their ready accessibility for far-UV radiation. Though they consist of a high proportion of strongly absorbing material, their small diameter (usually below 2 μm) permits the radiation to reach most parts of an individual cell equally well. In contrast, larger biological objects with a diameter of tens or hundreds of μm, though appearing small to the human eye, may be merely affected on the surface, and critical components inside may absorb little, if any, UV.

Of further considerable importance is the fact that some bacterial and viral nucleic acids can be irradiated in vitro, post-treated in various ways, and yet their biological function (as well as their photochemical alterations) tested. Examples are bacterial transforming DNA from *Haemophilus influenzae*, *Bacillus subtilis*, and other species, as well as various infective viral nucleic acids. With transforming DNA one considers essentially a small region of the macromolecule carrying a particular genetic marker that enters the competent (transformable) cell and is integrated into the recipient genome. In work with infective virus DNA one looks usually at the whole nucleic acid molecule, which under appropriate experimental conditions behaves like the complete virion, unless it is affected by the radiation. The experimental opportunities offered by such infectious nucleic acids systems will become evident in later sections.

As has been true for other areas in molecular biology, the use of bacteria and viruses, in particular phages, as model systems has played an essential role in the development of UV photobiology. Knowledge obtained from their study can now be successfully applied to problems encountered with more complex biological systems and structures. Among bacteria, by far the best-investigated species is *Escherichia coli*. In no other bacterium is the chromosome map known in such detail, or are so many different types of mutant derivatives available. Most UV studies with bacteria have been made using *E. coli* strains, and nowhere else is there as much detailed knowledge about repair processes after UV damage. Therefore, omission of specifying a species with which certain results are obtained usually implies that it is *E. coli*.

E. coli serves as a host for many types of phages. Some of them have become model viruses for UV photobiology and several other areas of molecular

biology. Phage T4 is certainly the best-investigated type of complex virulent phage, and the same is true for λ as a temperate phage. The information obtained about their UV effects has often been the basis for UV studies in other viral systems, particularly animal and plant viruses, where the expermental procedures involve greater difficulties. Similarly, the analysis of UV phenomena in *E. coli* cells, which has clarified the causal chain from UV absorption via photoproduct formation and repair processes to the finally observed biological effect, has been the basis for understanding the UV photobiology of other prokaryotic as well as eukaryotic cells.

2.2 UV radiation as experimental tool and environmental factor

There are two fundamental aspects to the application of UV radiation in biological experiments: (1) UV can be used as an *experimental tool*, which helps gaining insights into biological functions; (2) UV radiation, in the form of sunlight, constitutes a *natural environmental factor*, whose impact on biological systems (in a positive or negative sense) is of great interest. Each of these two aspects alone would have justified the efforts made to understand biological UV effects. Although the corresponding two emphases in the direction of research differ in principle, they complement each other, and the achievements and interpretations of experimental results often overlap.

As an experimental tool. The principle of using UV radiation as a tool is essentially this: Application of an external factor (UV) causes in cells or other biological systems temporary or permanent alterations that can be identified. They result from photochemical reactions in biomolecules, whose functioning can be assessed relative to the quality and quantity of damage inflicted in them. One might think that many other physical or chemical agents could serve the same purpose, but they usually lack some of the advantages associated with UV radiation. Ultraviolet light acts predominantly through photochemical alterations in DNA. Although other cell components may be affected, DNA is of prime importance because of its unique role as genetic material and its very high UV sensitivity (see Chapter 3). Within DNA, pyrimidine bases are essentially affected, with predominant formation of one group of photoproducts: the 5,6-cyclobutyl dipyrimidines (*pyrimidine dimers*). Quantitative assessment of their formation as a result of irradiation, and of their disappearance as a result of repair, is achieved by conventional chromatographic methods. Comparative studies in vitro with biologically active DNA (see Chapter 5) are valuable for complementing results obtained with whole cells or viruses.

Because of the central role played by DNA within the cell, these primary photo-effects can have quite a variety of biological consequences, in particular lethality, mutation induction, delay of growth or cell division, derepression, or enhanced genetic recombination. Even in heavily UV-damaged