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PHOTOMEDICINE

Volume I

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CRC PRESS

Photomedicine

Volume I

Editors

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INTRODUCTION

The use of sunlight and drugs for the treatment of skin diseases has been documented for over 3400 years; for an even longer time, the reddening, blistering, and tanning effects of sunlight have probably been known. With the discovery of lasers a new dimension was added in the study and application of light in medical therapy. Ophthalmologists adopted the laser in the clinic as a photocoagulator for the treatment of detached retina, and the use of laser as a scalpel for noncontact, noninvasive, and even subcellular surgery is at an earlier state of acceptance. In addition to surgical uses, new, promising ideas are continuing to emerge. Thus, laser can be used to diagnose and treat malignant tumors using photoradiation therapy. This renewed interest, stimulated by the mutual interplay of both scientific and technological innovations, is characterized by a multidisciplinary approach involving physicists, chemists, biochemists, and physicians.

Our objective has been to collect in these three volumes the most up-to-date assessment of our understanding of light in medicine. Since *Photomedicine* was defined as an informative guide to practical applications rather than an esoteric study of medical discipline, the level of medical rigor was reasonably relaxed.

Given limitations on length, the chapters are not intended to be all embracing reviews of the field, but rather to present an overview of key ideas and directions with the objective of delineating the most promising and exciting problems. We hope that the text is sufficiently introductory to stimulate the curiosity and interest of a neophyte, and to simultaneously provide the specialist with a rather short, but current summary of the status of this field. Most important, we hope that the volumes will further highlight this rapidly developing science and spur current and new researchers and ideas.

Ehud Ben-Hur
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THE EDITORS

Ehud Ben-Hur, Ph.D., was born in Israel in 1940. After graduation from the Hebrew University of Jerusalem in 1965, he went on to study biochemistry at the Technion, Israel Institute of Technology at Haifa, where he obtained his M.Sc. and doctorate degrees. He then joined the Biology Department of Brookhaven National Laboratory as Research Associate where he completed postdoctoral work on the radiobiology of cultured mammalian cells under the auspices of Dr. M. M. Elkind. Upon returning to Israel in 1973, he first joined the Department of Cellular Biochemistry at the Hebrew University and then the Nuclear Research Center-Negev, in 1975, where he is currently engaged in studies of biological effects of ionizing and nonionizing radiations.

The main thrust of his research activity in the past was related to radiation-induced damage in DNA and its repair. During the last few years he has become interested in photodynamic therapy of cancer and is actively involved with Dr. I. Rosenthal in developing new and improved photosensitizers for this purpose.

Dr. Ben-Hur is affiliated with the Department of Radiation Biology, Colorado State University. He is also affiliated with Ben-Gurion University, Beer-Sheva, Israel, where he teaches photobiology. Dr. Ben-Hur has published over 80 papers in scientific journals, is a member of the American Society for Photobiology and the Radiation Research Societies of both the U.S. and Israel, and is on the Editorial Board of the *International Journal of Radiation Biology*.

Dr. Ben-Hur is married with two children and lives most of the time in Beer-Sheva.

Ionel Rosenthal, Ph.D., received his degree in Chemical Engineering from the Polytechnic Institute in Bucharest (Romania) and Ph.D. degree from the Freinberg Graduate School of the Weizmann Institute of Science, Rehovoth, Israel. Dr. Rosenthal has had a very colorful professional career which has included Plant Engineer at "Mahteshim" Chemical Co. and Senior Scientist at the Department of Organic Chemistry at the Weizmann Institute of Science and at the Department of Organic Chemistry, Nuclear Research Center-Negev. Currently he is Principal Scientist at the Department of Food Science, Agricultural Research Organization, Bet-Dagan, and Professor in the Department of Agricultural Biochemistry at the Faculty of Agriculture of the Hebrew University, Jerusalem.

His scientific interests in organic photobiochemistry and food chemistry (and its spin-off: cookery) have resulted in more than 100 research publications in these areas.

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Chapter 1

BASICS OF PHOTOCHEMISTRY

Ionel Rosenthal

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I. PHOTOCHEMICAL TERMS

The science of photochemistry is concerned with the chemical and physical effects associated with electronic excitation of the chemical compounds. The promoter of excitation, for most practical purposes, is electromagnetic radiation in the UV and visible region of the spectrum (Table 1). Electromagnetic radiation can be regarded as having a dual nature.

• The light propagation phenomena such as reflection, refraction, interference, diffraction, and polarization can be explained in terms of a wave propagation. The wave is composed of oscillating electric and magnetic fields operating in planes which are perpendicular to each other and to the direction of propagation. The time variable strength of the resulting vector could be described by a sinusoidal function (Figure 1). The wave of radiation is characterized by wavelength (λ) which is the distance between identical positions on successive waves, or by frequency (ν) which is the number of waves that pass a fixed point per second. These two parameters are related in Equation 1:

$$\nu = \frac{c}{\lambda} \tag{1}$$

where c is the speed of electromagnetic radiation in vacuum (2.9979×10^8 m/sec). The wavelength of radiation determines the color of the light.

• At the same time, the phenomena of absorption or emission of radiation must occur only in discrete units called quanta or photons which have energies E as given by Equation 2:

$$E = h\nu = \frac{hc}{\lambda} \tag{2}$$

where h is a universal constant called Planck's constant (6.6256×10^{-34} J sec). It results that the energy absorbed or emitted by a molecule is inversely proportional to the wavelength of radiation. The energy of one "mole" of light, *viz.*, an Einstein, is given by Equation 3:

$$E = N \frac{hc}{\lambda} \tag{3}$$

where N is Avogadro's constant (6.022×10^{23}).

The first law of photochemistry — the Grotthuss-Draper law — requires that the radiant energy must be absorbed in order to induce a photochemical or photobiological reaction. The energies which can be absorbed by a particular molecule can be determined from the wavelengths in the absorption spectrum. The absorption spectrum of each molecule is, in turn, determined by the kind and arrangement of its component atoms. The first law of photochemistry is true for conventional light sources, but may require modifications for polyphotonic processes initiated by intense light sources such as lasers.

The second fundamental law, which is a result of the quantum theory, was proposed by Stark and Einstein and states that if a molecule absorbs radiation, then only one is excited for each quantum of radiation absorbed. The Stark-Einstein law implies that the efficiency of a photochemical process can be defined by its quantum yield (ϕ) which is the ratio of molecules undergoing a particular change and the number of quanta absorbed. In most photochemical reactions, the quantum yield will range from zero to

Table 1
THE DISTRIBUTION OF THE ELECTROMAGNETIC SPECTRUM

Radiation	Wavelength	Absorption or emission of radiation involves
Gamma rays	0.0005—0.14 nm	Nuclear transition
X-rays	0.01—10 nm	Transitions of inner atomic electrons
Vacuum UV	1—200 nm	Transitions of outer atomic electrons
UV	200—400 nm	Transitions of outer atomic electrons
Visible	400—800 nm	Transitions of outer atomic electrons
Near IR	0.8—1.5 μm	Molecular vibrations
IR	1.5—5.6 μm	Molecular vibrations
Far IR	5.6—1000 μm	Molecular rotations
Micro- and radiowaves	1000 μm—550 m	Oscillations of mobile or free electrons
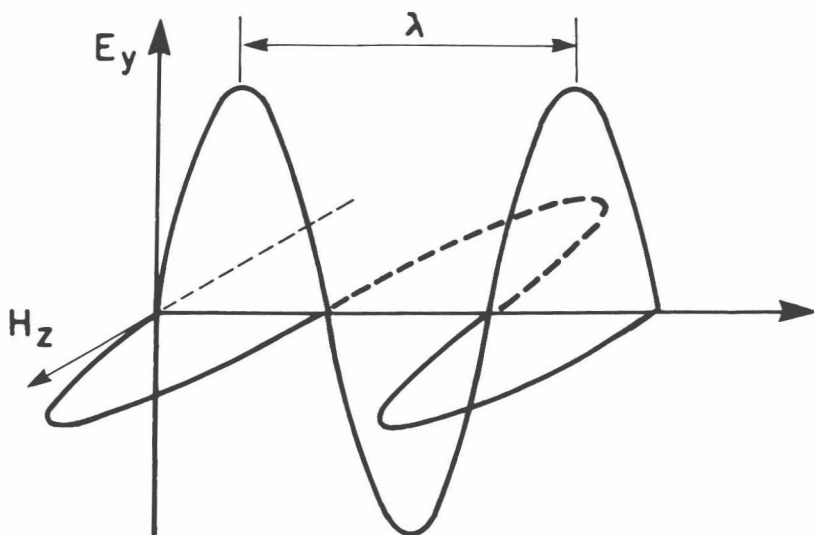
FIGURE 1.   The electric (Ey) and magnetic (Hz) vectors of an electromagnetic light wave.

unity. However, in a chain reaction, where the absorption of one photon initiates the reaction, the value of the quantum yield may be several powers of ten. The determination of the quantum yield of a process will depend on the accurate determination of the number of molecules reacting or formed, and the number of photons absorbed. While the first quantity can be obtained by chemical analyses, a measure of the number of photons absorbed is best achieved by comparison with a reaction system, the quantum yield of which has been determined accurately using a photosensitive instrumental technique. Such a reaction system is known as an actinometer.

The intensity of an absorption band follows the empirical Beer-Lambert law, which states that the fraction of light transmitted through an absorbing system is as presented in Equation 4 and, in the log form, in Equation 5:

$$\frac{I_t}{I_o} = 10^{-\varepsilon c d} \tag{4}$$

FIGURE 2.   Energy level (Jablonski) diagram indicating the principal light-induced molecular photophysical processes. VR = vibrational relaxation, 10^{-10} sec; IC = internal conversion, 10^{-10} sec; ISC = intersystem crossing, 10^{-6} sec.
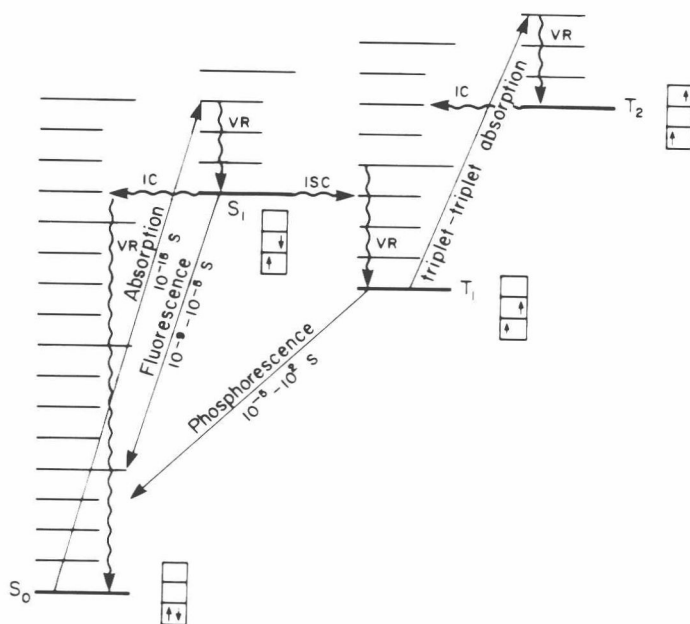
$$\log \frac{I_o}{I_t} = \xi cd \tag{5}$$

where I_t and I_o are transmitted and incident light intensities, c is the concentration of the absorber, and d is the depth of absorber through which the light has passed. ξ is a constant of proportionality known as the extinction coefficient and is dependent on the wavelength and occasionally on concentration. The product ξcd is also named the optical density (OD) of the system.

When a molecule absorbs energy, the process is referred to as excitation. It is possible to represent the various physical processes involved in the photochemical excitation in the Jablonski diagram (Figure 2). The molecule is raised from its ground state of minimum energy to an excited state of higher energy. In the UV and visible region of the electromagnetic spectrum, this involves excitation to electronic energy levels. In the IR region, the excitation occurs between rotational levels at the lower energies of the far IR, while increase in the vibrational energy occurs in the near and medium IR region. While it is generally true that electronic excited states are the precursors of a photochemical change and, as such, are of primary importance in photobiology, they are inevitably accompanied by some increase in vibrational and rotational energy.

The electronic structure of the absorbing molecule changes as the result of excitation. This change is visualized in simple molecule orbital terms as a change in the occupation pattern of a set of orbitals. The combination of two atomic orbitals of different atoms by overlap along the internuclear axis results in the formation of two molecular orbitals — a bonding orbital (either σ or π), and an antibonding orbital (σ^* or π^*). In addition, the nonbonding electrons, if present as at an oxygen atom, occupy a nonbonding orbital n which by its nature does not possess an antibonding counter-

part. Two electrons are assigned to each molecular orbital so that their spins are paired. In general, a molecule in its ground state has all of its electrons spin-paired (a notable exception is molecular oxygen, the ground state of which is a triplet). The absorption of a quanta of radiation in the UV-visible range by a molecule leads to electronic excitation of the absorber. That is the promotion of an electron from one molecular orbital to another, unoccupied, of higher energy, e.g., $\sigma \to \sigma^*$, $n \to \sigma$, $\pi \to \pi^*$, and n $\to \pi^*$. In general, excitation of an electron to the σ^* orbital requires an amount of energy corresponding to wavelengths below 200 nm, which are not readily accessible to conventional irradiation apparatus and are of little relevance to photobiology. The alternative excitation processes ($\pi - \pi^*$ and $n - \pi^*$) are responsible for the bulk of photochemical reactions, since the wavelengths associated with these two excitations are located in the accessible regions of the UV and visible part of electromagnetic spectrum.

The initial electronic excitation occurs without change in the orbital spin; i.e., the molecule, although in the excited state, still preserves the "singlet" multiplicity. The multiplicity, M, of a system is defined by Equation 6:

$$M = 2S + 1 \tag{6}$$

where S is the total spin of the system. Thus, in a spin-paired system, $S = 0$ and $M = 1$ (singlet), while for a spin-parallel system, $S = 1$ and $M = 3$ (triplet). The excited singlet state is very short-lived and it may decay to the original ground state by conversion to heat or spontaneous photon emission (fluorescence). Alternatively, the molecule in this state could be destabilized to initiate a chemical reaction or could convert to a triplet state in a process which involves a spin flip of one of the electrons in the highest occupied molecular orbital. In this process, named intersystem crossing, the system becomes spin-parallel. Since transitions between states of different multiplicities are "forbidden" because they involve a spin inversion, the reversal from the excited triplet state to the ground singlet state is slow, and the molecule in the triplet state is long-lived. A triplet state always has a lower energy than the corresponding singlet state because of the repulsive nature of the spin-spin interaction between electrons of the same spin. The spontaneous radiation emission from the triplet state is known as phosphorescence. Because of its long lifetime, the triplet-state molecule is the most important in the initiation of chemical and biochemical reactions in vivo and in vitro. Reactions may also originate from excited singlets, but their much shorter lifetime most often limits their importance. The triplet molecule may react directly, inter- or intramolecularly, or may dispose of its energy by energy transfer to a vicinal suitable system. The concept of energy transfer can be represented in a general form by Equation 7:

$$^3D^* + {}^1A \to {}^1D + {}^3A^* \tag{7}$$

where D and A are the donor and acceptor, respectively, in the triplet and singlet states. Excitation of a ground-state molecule by energy transfer from another excited species is termed sensitization, and the deactivation of the excited species is termed quenching. The energy-transfer processes play an important role in photobiology. Thus, the photosensitization creates the possibility of generating an excited molecule indirectly, rather than by its irradiation. Furthermore, the excited states of many organic molecules are quenched by molecular oxygen in a diffusion-controlled manner. Consequently, photochemical reactions are often drastically altered by the presence of molecular oxygen.

II. THE PRINCIPLE OF LASING

The laser (*L*ight *A*mplification by *S*timulated *E*mission of *R*adiation) is a device by which interaction of a photon with an excited species induces emission of a photon from this species. As the name implies, the operation of a laser depends on a stimulated emission of radiation. Stimulated emission is rarely important in photochemical processes in which thermal equilibrium is established. However, in a nonequilibrium situation, when the spontaneous emission in the system is suppressed in some way, the excited state becomes more populated than the ground st..te ("population inversion"). At this stage, the absorption of a photon of the transition frequency will trigger an emission which will predominate over absorption, and a net emission will result. The lasing system consists of an emitting material set between a pair of parallel mirrors, one of which is partially transparent. The mirrors are separated by an integral number of half of the emission wavelength. As a result, the light reflected from a mirror will be in phase with the incident wave, by a process called constructive interference. The population inversion essential to laser action is achieved by excitation to an excited state which is higher in energy than the lasing state. The excitation is most often achieved by photochemical techniques. When a population inversion has been generated in the emitting material, spontaneous emission provides a few photons, which on collision with molecules of the exciting lasing material stimulate it to emit in phase with the incident photons. Thus a light wave builds up in intensity as it travels back and forth between the mirrors, and this cascade reaction destroys the population inversion in a very short interval of time (1 μsec) producing a burst of radiation which escapes through the partially transmitting mirror. Since the cavity is tuned only for axial radiation, all off-axis radiation escapes after few reflections without reaching the lasing stage. Subsequently the laser beam exhibits a very accurate parallelism and can be focused to a spot of very small dimensions (of the order of a wavelength), thus generating very high radiation densities ($>10^9$ W/cm²) for short laser pulses. In addition, the cavity is tuned to one particular frequency, and the light of any other frequency is removed by destructive interference. Subsequently, the emerging light exhibits a very high monochromaticity and coherence; i.e., the emitted light waves are all in phase.

III. QUANTUM LIGHT UNITS OF MEASURE

Many different terms and units have been, and still are being used in the literature to describe light. Several slightly different values can be found in the literature for conversion factors. The origin for discrepancies could not always be traced. The conversion factors between various units of energy of electromagnetic radiation are as follows:

$$1 \text{ J} = 10^7 \text{ erg} = 6.25 \times 10^{18} \text{ eV} = 2.38 \times 10^{-4} \text{ kcal}$$

The intensity of light is expressed in units of energy per unit of cross section. Most commonly, W/cm² or related energy units are employed. When using this term, the wavelength region must be specified. Alternatively, Einstein/sec/cm² is also used for radiant energy in a mole of photons.

The intensity of a source of visible light can also be measured in candle power. The candela (candle) is 1/60 of the luminous intensity of 1 cm² of a full radiator at the freezing temperature of platinum (2046 K). More recently, candela was defined as the luminous intensity of a source emitting monochromatic radiation with a frequency of 540×10^{12} Hz and a radiant intensity of 1/683 W per steradian. This corresponds to about 54.5 candle power to 1 W at 556 nm, which is the wavelength of maximum