

Photoimmunology

Edited by John A. Parrish · Margaret L. Kripke · Warwick L. Morison

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Preface

In this book we attempt to summarize the present state of knowledge in the field of photoimmunology and to provide the background necessary to put this knowledge into perspective. This objective requires descriptive overviews in photobiology, immunology, and dermatology. Our hope is to facilitate interdisciplinary communication and to stimulate interest in photoimmunology.

Section I, Principles of Photoimmunology, describes (1) basic interactions between UV and visible radiation and biologic matter, (2) the optical properties of human skin, and (3) normal and abnormal responses to UV radiation. Basic principles of selected aspects of immunology and photobiology are explained. Section II, Experimental Photoimmunology, describes molecular, cellular, animal, and human studies in photoimmunology. Section III, Clinical Photoimmunology, presents evidence of the involvement of photoimmunologic mechanisms in diseases of the skin and blood and examines the possible involvement of immunologic mechanisms in therapeutic photomedicine. The fundamental principles outlined in this book should prove useful for decades to come. However, our expectation is that the experimental data will be outdated in a short period of time. The potential of photoimmunology is great enough and the serious study of photoimmunology is recent enough that this should be the case. This book is dedicated to the goal of continuing rapid growth in the field of photoimmunology.

John A. Parrish
Margaret L. Kripke
Warwick L. Morison

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Section I

Principles of Photoimmunology

Human responses to solar radiation are complex and affect not only skin and eyes but also circulating and noncirculating components of the immune system. Photoimmunology, the study of the effects of light on the immune system, is an intersection of photobiology, dermatology, and immunology. In certain cutaneous diseases, manifest by photosensitivity, radiation within the UV and visible wavebands alters immune reactions in skin. Several skin and blood diseases that are thought to have immunologic pathogenesis can be treated successfully by UV radiation. UV-radiation-induced alterations of immune function play an important role in the pathogenesis of UV-radiation-induced skin cancer in mice. Today it is recognized that tolerable exposures of the skin of experimental animals and human volunteers to UV radiation can diminish allergic contact dermatitis (contact hypersensitivity), diminish delayed-type hypersensitivity to an injected antigen, prolong skin-graft survival, and alter the function and distribution of lymphocytes.

Humans have a complicated, dependent relationship with the sun. All life derives its energy from the sun; photosynthesis drives food chains and the radiant energy of the sun is the major source of heat. Vitamin D is synthesized in the skin by a photochemical reaction involving UV radiation from the sun. Visible light photochemistry in the retina provides a convenient instantaneous map of our environment. On the other hand, excessive exposure of skin to the sun causes cell injury and inflammation, mutation and cancer, and degeneration of connective tissue.

Skin is the largest organ of the human body and is readily available for observation and study. The skin serves to protect man from a noxious environment and to maintain a homeostatic internal milieu. It shields us from chemicals and bacteria and absorbs most of the physical stresses of our world. Acting as an insulator and a membrane, the skin keeps the environment within the body at a relatively constant temperature and with a stable water and salt content. The skin is the first barrier against photons and is the site of the initial steps of

2 Section I

photoimmunologic responses. During evolution, the skin of mammals developed optical and chemical mechanisms for protection against UV radiation and limited physiologic capacity for increasing that protection.

In some aspects, the skin can also be considered an immune organ. Experimental immunologists have looked at skin primarily as a medium for the display of immunologic reactions. It has been used to study delayed-type hypersensitivity, the Arthus reaction, and the allograft response. Recent studies have shown that the skin is an active component of the immune system and that lymphocytes interact with the skin in a manner that affects their subsequent physiologic and pathologic performance. Phylogenetically, ontogenetically, and anatomically, there is a strong association between lymphocytes and epithelium. Certain lymphocyte malignancies, such as T-cell lymphoma and mycosis fungoïdes, have strong affinities for skin and can be treated by inducing cutaneous delayed-type hypersensitivity. Lymphocytes migrating in skin may be the bearers of transplantation antigens within skin and graft-versus-host reactions may be directed at these lymphohematopoietic cells residing or passing through skin. In some species, the skin contains a population of cells that is immunocompetent and, in some experimental systems, the skin retains immunologic memory. The microenvironment of the skin is sufficient to allow antigen-reactive lymphocytes to perceive foreign or altered antigens *in situ* and to react against them. Several skin diseases have pathophysiologic mechanisms such that the signs, symptoms, and histopathologic changes result from immunologic reactivity.

Ultraviolet, visible, and near-infrared radiation enters into and is absorbed by the skin and the cutaneous blood and can initiate major alterations in molecules and cells. Considering its metabolic needs, the skin is highly overperfused with an average blood flow 20 to 30 times the minimum flow needed to supply skin cells. This is because the cutaneous vasculature is a heat-regulatory system for the organism as a whole. Blood, equivalent to the whole blood volume, passes through the skin every few minutes. Thus, UV radiation can reach the superficial vasculature in sufficient amount to alter the viability and function of circulating blood cells. There are various thermal, pharmacologic, and physiologic techniques that can alter blood flow to increase or decrease the amount of blood available for *in situ* UV radiation.

Photons are potentially very useful tools for uniquely studying the immune response. Nonionizing electromagnetic radiation within the UV and visible range is capable of selectively altering specific molecules within complex systems without altering other nonabsorbing molecules. The photochemical alterations can lead to very specific and identifiable changes in cell metabolism and function. Certain cells, such as Langerhans cells, and certain immune processes, such as antigen presentation, are selectively sensitive to UV radiation. Subpopulations of cells vary in their sensitivity to UV radiation. Therefore, manipulation of exposure dose parameters may allow isolation and study of specific aspects of immune function.

Radiation in the 320- to 800-nm waveband has properties that may make this waveband useful for the purpose of selective *in vivo* photochemistry utilizing exogenously supplied chromophores. This radiation is not very phototoxic. It has sufficient quantum energy to initiate photochemical reactions, yet is relatively innocuous to exposed human tissues other than the eye. Ultraviolet and visible radiation transmitted into skin and cutaneous blood vessels has an internal dosimetric depth profile; in soft tissue, penetration of this radiation is very wavelength dependent because of the optical properties of the tissue.

Any of the responses of human tissue to UV radiation that can be identified and quantified can be induced by artificial sources and can therefore be manipulated in a variety of ways. Identification and quantification of photon-induced responses to the immune system may lead, through intelligent creative thinking, to therapeutic manipulations of the immune system. This aspect of photoimmunology is poorly defined but has exciting potential. Ultraviolet-radiation-induced immunologic effects may explain the benefits of many present forms of phototherapy and photochemotherapy. Successful treatment of atopic eczema, mycosis fungoides, and lichen planus serve as a few possible examples. Ultraviolet radiation could be used to manipulate the immune system for beneficial alteration of host defenses, treatment of allergic responses, or induction of specific immune tolerance. For instance, specific wavebands of UV radiation affect animals systematically so that subsequent exposure to an antigenic substance induces suppressor T cells and diminishes sensitization capacity specific for the particular antigen. Photosensitizing chemicals could greatly enhance the potential for selectivity of these effects.

This section discusses basic principles of photobiology as they apply to dermatology and immunology. Chemical photosensitization will be discussed in detail because certain drugs used to enhance the effects of UV and visible radiation on mammalian cells may be useful to increase specificity in studying or manipulating the immune system. The optical properties of skin influence the cells and components of the immune system that are affected by UV radiation. *In vivo* photoimmunology studies and therapeutic applications must utilize exposure doses tolerable to the skin. Therefore, the acute effects of UV radiation upon skin will also be discussed in some detail, with emphasis on the inflammatory response to photon-induced cell injury. A survey of photobiologic and experimental factors influencing photocarcinogenesis provides the background to understand the role of photoimmunology in the etiology, pathogenesis, and progression of skin cancer.

J.A.P.

Chapter 1

Basic Concepts in Photobiology

Irene E. Kochevar

1.1. Introduction

Photobiology encompasses many diverse subjects: Photomedicine, vision, photosynthesis, bioluminescence, and others. The effect of nonionizing radiation on biologic systems, as well as the use of these wavelengths to study biologic phenomena, constitutes photobiology. Photomedicine, which includes photoimmunology, is concerned with the effect of nonionizing radiation on the human organism. The areas of photomedicine share a common pathway from the absorption of nonionizing radiation by molecules in the system to the observable biologic effects. This pathway is depicted in Fig. 1.1. In the first step, radiation is absorbed by a molecule in the biologic system, such as DNA, protein, or porphyrin. The light-absorbing molecule is referred to as a chromophore. Specific chromophores are present for each photobiologic response. After absorbing the energy of the radiation (electromagnetic energy), the molecule is in an excited state. This form exists for only a fraction of a second. In the second step, the excited-state molecule undergoes a chemical change to form a photoproduct. The photoproduct may result from rearrangements within the molecular framework of the chromophore or from reaction of the chromophore with another molecule.

Creation of photoproducts in cells may initiate complex biochemical processes (Step 3) involving more than one reaction, for example, enzymatic repair, generation of ion fluxes, induction or inactivation of enzymes, and initiation of DNA replication. These biochemical responses culminate in cellular effects (Step 4), such as proliferation, mutagenesis, loss of cell surface markers, and toxicity. In the final step, an organ or the whole organism may be involved, and a photobiologic effect (Step 5) is manifest as erythema in skin, appearance of hyperplasia or tumor, vision, plant growth, and many other phenomena. Descriptions of each of these steps as they apply to photoimmunology form the basis of this chapter.

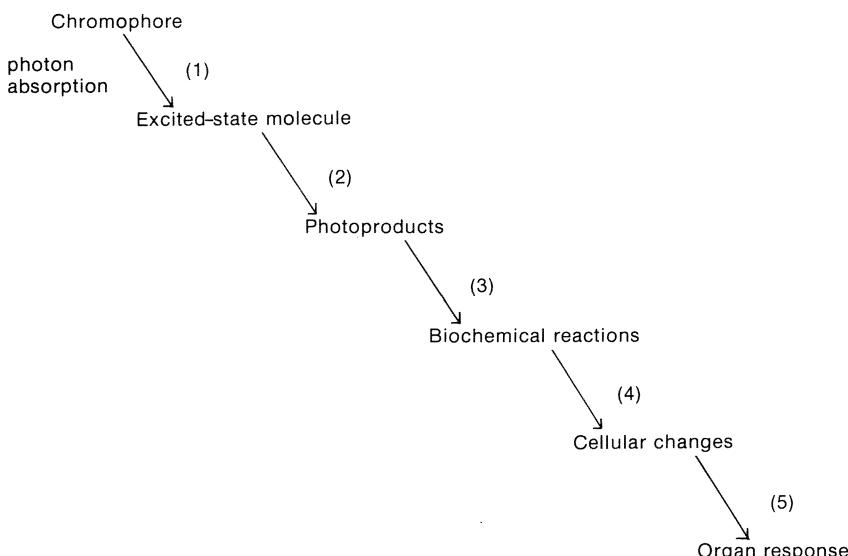


Figure 1.1. A scheme showing steps in the response of a biologic system to light. (1) Light is absorbed, (2) photochemistry occurs, (3) the photoproducts elicit a biochemical response that is reflected as a (4) cellular and (5) organ response.

1.2. Absorption of Electromagnetic Radiation and Characterization of Excited-State Molecules

The first step of the general photobiologic scheme requires the absorption of nonionizing radiation by molecules in the biologic system.^{1–3} The characteristics of molecules and light, which are essential for understanding this interaction, are presented.

1.2.1. Electronic States of Molecules

In simplest terms, organic molecules consist of nuclei, which are in relatively fixed positions, and electrons, which are found in defined volumes surrounding the molecular structure. For each compound, a series of electronic states exists, each of which corresponds to a certain spacial distribution of electrons around the nuclei. Associated with each electronic state is a molecular energy. According to quantum mechanics, only certain energies are allowed; thus, fixed energy gaps exist between electronic states. At room temperature, almost all molecules are in the electronic state with the lowest energy, the ground state (Fig. 1.2). Molecules in states with higher energy are in excited electronic states. Energy can be absorbed by molecules in a lower electronic

state to promote them to a higher energy electronic state. In reality, the nuclei are not immobile. The motions of the nuclei are called vibrations and rotations, and the energy of the vibration and rotation states is also quantized, i.e., can have only fixed values. Many vibration and rotation states are associated with each electronic state as shown in Fig. 1.2.

The electrons in a molecule occupy orbitals, which are specific regions of space around the nuclei. Each orbital can hold a maximum of two electrons. In ground-state molecules, the orbitals almost always contain two electrons. Electrons have a property called spin. According to the Pauli exclusion principle, electrons in the same orbital must have opposite spins. The spins of the electrons can be used to calculate a characteristic called the multiplicity:

$$\text{Multiplicity} = 2S + 1 \quad (1)$$

where S = absolute value of the total electronic spin. The photochemical reaction of an excited state is partially dependent on its multiplicity. Multipli-

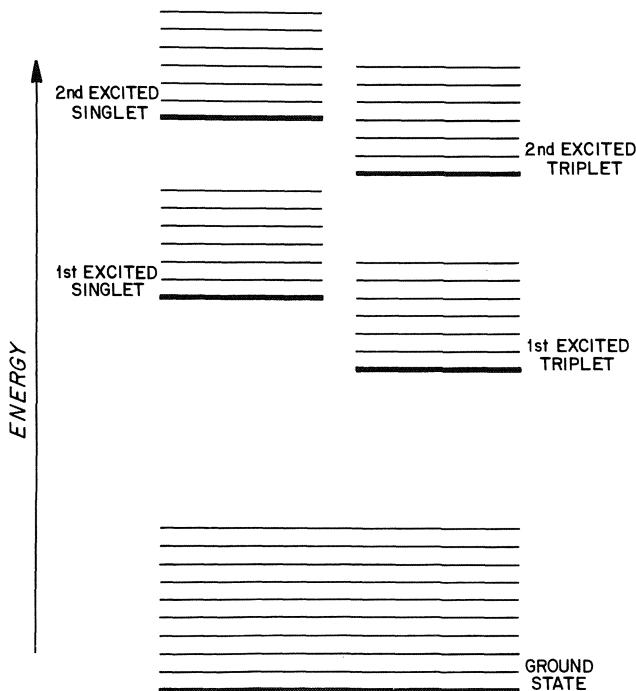


Figure 1.2. Schematic diagram of the electronic, vibration, and rotation energy states of a molecule. The heavy lines represent the energy levels of the electronic states. The light lines represent the energies of the vibration and rotation states. Molecules can change from one state to another by absorbing or losing energy.

cities of 1 (singlet state) and 3 (triplet state) are found for organic molecules. The value of S is based on using $+1/2$ and $-1/2$ for the two opposite spins of electrons. When the molecule contains an equal number of electrons with each spin, $S = 0$ and the multiplicity is 1 (singlet state). Most ground-state molecules have two electrons in each orbital and, therefore, must be singlet states. The oxygen molecule is a notable exception. It exists as a ground-state triplet. Both excited singlet states and excited triplet states exist for organic molecules. The triplet has a lower energy than the singlet in each electronic state because of lower electron-electron repulsion in triplet states. Molecules exist in excited singlet and triplet states after light absorption. This process is described later in this chapter.

1.2.2. Electromagnetic Radiation

Electromagnetic radiation (EMR) is a form of energy. The interaction of EMR with matter can be described by viewing it either as a continuous wave (wave description) or as a series of packets containing energy (particle description). Each description is best applied to a particular phenomenon. Transmission through space, scattering, and diffraction can be best understood using the wave description. Here, EMR can be characterized as a continuous wave of regular oscillations of electric and magnetic fields. These fields are perpendicular to each other and to the direction of propagation. However, absorption of EMR by molecules and the photoelectric effect are best understood when the particle description of EMR is used. In the particle description, EMR is contained in discrete packets, called photons.

The energy of EMR is directly proportional to the frequency, ν , of the oscillation of the two fields:

$$E = h\nu \quad (2)$$

where E = energy of a photon, h = Plank's constant (6.63×10^{-34} J/sec), and ν = frequency. The product of the frequency and the wavelength is equal to a constant, the speed of light in a vacuum:

$$\nu\lambda = c \quad (3)$$

where λ = wavelength and c = speed of light in a vacuum. Therefore, the energy of EMR is inversely proportional to its wavelength:

$$E = \frac{hc}{\lambda} \quad (4)$$

The term *frequency* is often used in spectroscopic studies, whereas the term *wavelength* of the radiation is more commonly used in photochemistry and photobiology.

The unit most often used for wavelength in the UV and visible range is the nanometer, which is 1×10^{-9} m. The electromagnetic spectrum extends from very long wavelength radiation (low energy), such as radiowaves ($\lambda \approx 3 \times 10^8$ m), to a very short wavelength radiation (high energy), such as cosmic rays ($\lambda \approx 3 \times 10^{-19}$ m). The EMR reaching the earth from the sun contains wavelengths from about 290 to 4000 nm. This radiation is described as UV, visible, or infrared, depending upon the wavelength (Fig. 1.3). The UV portion of the electromagnetic spectrum covers the range from 200 to 400 nm. Solar radiation shorter than about 290 nm is absorbed by ozone in the stratosphere and does not reach the earth's surface. The range from 200 to 400 nm is often arbitrarily divided into UVC, UVB, and UVA radiation (Fig. 1.3), based on skin reactions in humans. The UVA portion (320 to 400 nm) is longer-wavelength UV radiation, which is not strongly absorbed by protein and nucleic acids and does not cause erythema in normal skin at moderate doses in the absence of photosensitizing chemicals. This range is also called black light and near-UV radiation. UVB radiation (290 to 320 nm) is erythemogenic and is present in the terrestrial solar spectrum. It is also referred to as sunburn radiation and mid-range UV radiation. UVC radiation (200 to 290 nm) is biologically active but does not reach the earth's surface. However, the 254-nm line in low-pressure mercury lamps (germicidal lamps) is frequently used in experiments as a source of UV radiation. The spectral sensitivity of human skin to UV radiation is discussed in Chapter 7. All three regions of UV radiation affect cells of the immune system.

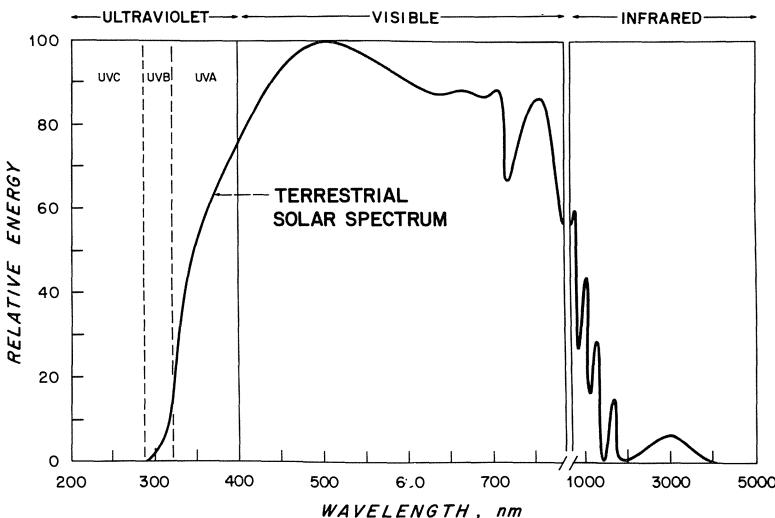


Figure 1.3. Spectrum of electromagnetic radiation that reaches the earth's surface from the sun. Wavelengths shorter than about 290 nm are absorbed by ozone in the stratosphere.

1.2.3. Absorption of Electromagnetic Radiation

For light to cause a chemical change, it must first be absorbed by molecules. In this process, the electromagnetic energy is converted to chemical bonding energy that is contained within the molecule. The photon no longer exists, and its energy is stored in the molecule. Absorption of a photon promotes a molecule to a higher electronic state, such as the first excited singlet state in Fig. 1.2. Radiation that reaches a biologic system, but is not absorbed, cannot cause a photobiologic response. In the absorption process, the entire energy of a photon is taken up by the molecule. The energy of the photon must match the difference in energy between the ground and excited electronic energy levels plus any changes in vibration and rotation energies.

The change in the electrons of a molecule produced by light absorption can be approximated by considering only one pair of electrons (Fig. 1.4). In the ground state, both electrons are in the same orbital. The electrons have opposite spins (singlet state). Absorption of a photon promotes an electron to the next higher orbital. No change in electron spin occurs; therefore, this electron distribution is an excited singlet state. If the higher energy electron changes its spin, its energy is lowered. This electronic configuration is an excited triplet state.

Molecules remain in electronically excited states until they undergo a chemical change to form a photoproduct, transfer the energy to another molecule, or shed their excess energy as either light or heat. Excited states are very brief. Excited singlet states usually last less than 100 nsec (10^{-7} sec) and excited triplets less than 1 sec.

Each absorption process occurs with a probability that depends on the structure of the molecules involved. The absorption spectrum is therefore a

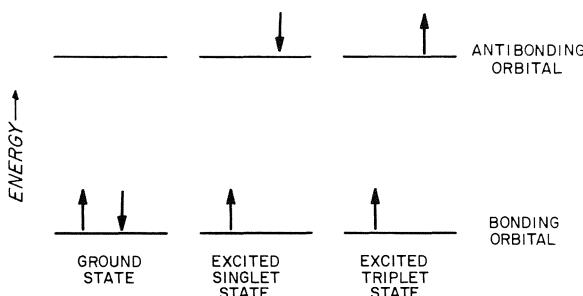


Figure 1.4. Schematic diagram of the distribution and spins of the electrons in one bond of a molecule. In the ground state, the electrons are in the bonding orbital and have opposite spins. In the excited singlet state, one electron has been promoted to the antibonding orbital, thus weakening the bond. The excited triplet state also has an electron in each orbital, but their spins are the same.

plot of the probability of absorption (ordinate) versus wavelength (abscissa). Fig. 1.5a represents an absorption spectrum of an isolated atom. Since no vibrations and rotations are possible, absorption occurs only at the specific wavelengths that correspond to the energy differences between electronic state energy levels (Fig. 1.2). When atoms are covalently bonded to form a molecule, many absorption processes occur. Transitions from the rotation and vibration levels of the ground state to the levels of the excited state are possible. The probability of each transition depends on the energy separation, the nuclear positions, and other factors. The absorption spectrum of such a molecule is made up of numerous lines representing the absorption probability of each wavelength. The lines are so close together, however, that they cannot be distinguished, and only the contour of the heights is recorded (Fig. 1.5b). The absorption spectra of biologic molecules are discussed in Chapter 2.

The probability that a photon will be absorbed by a chromophore is expressed by the molar extinction coefficient, ϵ . The value of ϵ varies with wavelength. Experimentally, the absorbance, A , of a solution containing the chromophore is measured. A is related to the extinction coefficient by the Beer-Lambert Law:

$$A = \epsilon lc \quad (5)$$

where A = absorbance, ϵ = extinction coefficient, l = length of the optical path, and c = concentration. A plot of A or ϵ versus wavelength is the absorption spectrum of a compound.

The wavelengths that have the highest probability of absorption are called absorption maxima, λ_{\max} , and are often used as identifying characteristics of a compound. The absorption spectrum of a compound is a function of the molecular structure of the compound. Compounds that have a large energy separation between the ground and lowest excited states (see Fig. 1.2) absorb short wavelengths because energy is inversely proportional to wavelength. The absorption is in the UV range for these compounds and they are therefore visually colorless. For example, for cholesterol, $\lambda_{\max} < 200$ nm, and for tryptophan, $\lambda_{\max} = 280$ nm. Organic compounds containing unsaturated groups, heteroatoms, or charged groups in their structures absorb longer wavelengths of light and may be visibly colored. The energy gap between the ground and excited states is smaller for these compounds. Examples are hemoglobin ($\lambda_{\max} = 410$ nm), and β -carotene ($\lambda_{\max} = 457,466$ nm).

The action spectrum for a photobiologic response, in an ideal case, corresponds to the absorption spectrum for the chromophore. The determination of action spectra is therefore a powerful tool for identification of putative chromophores. An action spectrum is a plot of the reciprocal of the number of incident photons required to produce a given effect versus wavelength. If photons in

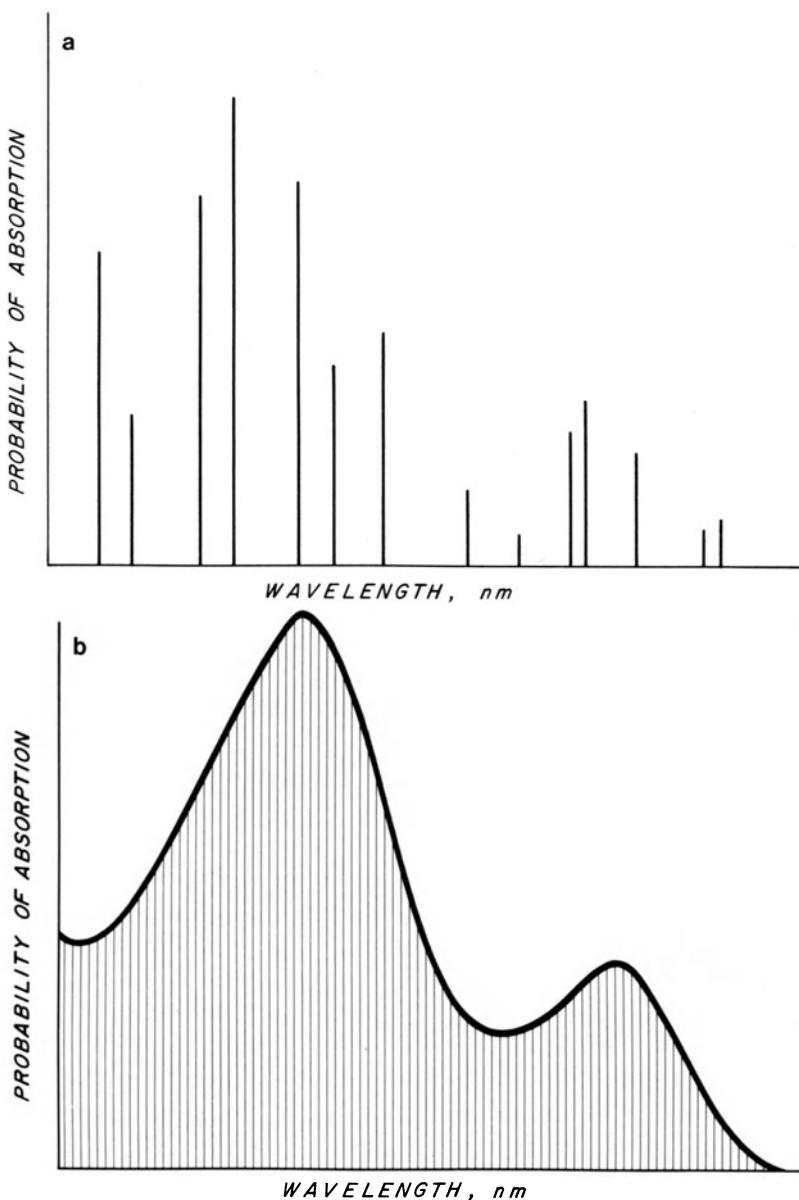


Figure 1.5. (a) Absorption spectrum of an isolated atom. Absorption occurs at specific wavelengths that represent the energy differences between energy states allowed by quantum mechanisms. (b) Absorption spectrum of an organic molecule in solution. Absorption occurs at many wavelengths because of the rotation and vibration levels associated with the electronic energy states.

a certain wavelength range are particularly effective, fewer photons are needed in that range than in other ranges. Peaks in the action spectrum correspond to the most effective wavelengths. Lack of correspondence between an experimentally determined action spectrum and the absorption spectrum of the actual chromophore may occur. Light that impinges on a tissue is usually absorbed by other chromophores in the tissue; it is also reflected and often scattered. These effects decrease the amount of light transmitted to a specific depth in tissue and vary with wavelength. Consequently, caution should be exercised when attributing a photobiologic effect to a chromophore in the biologic system. This problem has been reviewed.⁴ An action spectrum for inhibition of contact allergy by UV radiation is discussed in Chapter 9.

1.3. Reactions of Excited-State Molecules to Form Photoproducts

A molecule in the excited state has more energy and a different distribution of electrons than it does in the ground state. The positions of the nuclei are usually only slightly different. Electronically excited molecules can undergo reactions that were not possible in the ground state. The fate of an excited-state molecule depends on its environment (e.g., temperature, pH, solvent, and presence of other molecules).

1.3.1. Alternative Processes Available to Excited-State Molecules

An excited-state molecule can discharge its energy by chemical reaction to form photoproducts,⁵ by emission of light, by decay to heat (internal conversion), by transfer to another molecule, and by a change from singlet to triplet or from triplet to lower-energy singlet (intersystem crossing) (Fig. 1.6). The pathway that is followed varies from compound to compound and, for each compound, varies with its environment. Chemical reactions, which are discussed more thoroughly later in this chapter, are particularly interesting because they initiate almost all photobiologic responses. Emission of light is called fluorescence when it originates from a singlet excited state and phosphorescence when it originates from a triplet excited state. When a molecule has almost the same arrangement of nuclei in the excited singlet state as in the ground state, the fluorescence and absorption spectra partially overlap (Fig. 1.7), but the fluorescence extends to longer wavelengths. Because the energy gap between the ground state and the triplet state is smaller than that between the ground and the excited singlet state, the phosphorescence is at longer wavelengths (lower energy) than the fluorescence. Fluorescence is more commonly seen than phosphorescence. The environment of the excited-state molecule may affect the emission spectrum and yield. Consequently, fluorescence can be used to char-

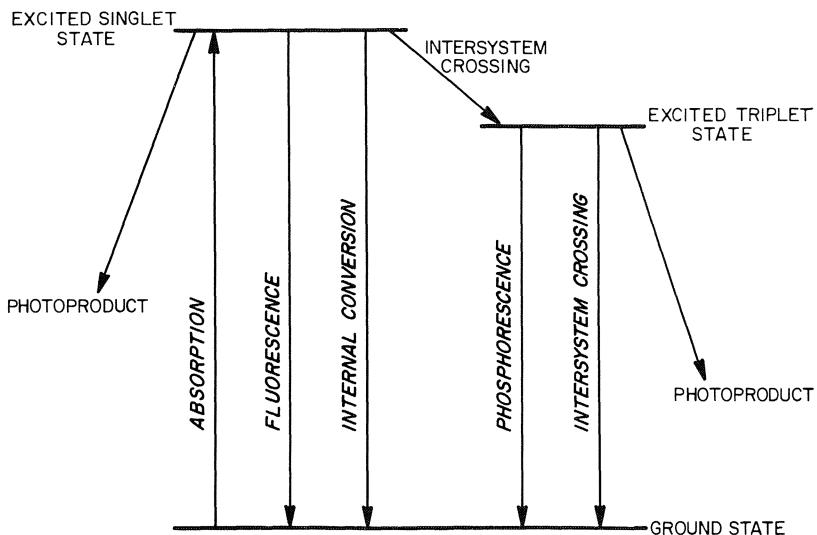


Figure 1.6. Schematic diagram of processes available to excited states. Absorption, fluorescence, and phosphorescence involve radiation loss or production. The rest are radiation-less processes. Internal conversion is a singlet-to-singlet transition involving loss of energy. Intersystem crossing is a singlet-to-triplet or triplet-to-singlet process. Transfer of electronic energy from both the singlet and the triplet states to another molecule is also possible but not shown here.

acterize molecular environments in biologic systems. The internal conversion pathway for loss of electronic excitation energy proceeds by conversion of the electronic energy into vibration energy within the molecule. This energy is quickly transferred to solvent molecules and, if enough is transferred, heat may be detectable.

In the electronic energy transfer process, an electrically excited donor molecule (D) is demoted to a ground state and the acceptor (A) molecule is promoted to an excited state.⁶ This process is described in the following equation, where the asterisk denotes the excited state:



Simultaneous electronic transitions occur. Emission of a photon by the donor and absorption by the acceptor are unlikely. The electronic energy of the acceptor molecule must be the same or less than that of the donor for efficient energy transfer. Transfer involving molecules in singlet excited states can occur over distances as large as 100 Å. Energy transfer involving molecules in triplet

excited states requires a closer approach, 10 to 15 Å. Energy transfer is one mechanism for photosensitized reactions.

For any given molecule in an excited state, the distribution of the electrons and the availability of another molecule with which to react determine which process (light emission, energy transfer, internal conversion, or reaction) will dominate. The term quantum yield is used to define the likelihood of these processes:

$$\text{Quantum yield} = \frac{\text{Number of molecular events of interest}}{\text{Number of photons absorbed}} \quad (7)$$

The concept of quantum yield must be extended further to make it applicable to photobiology because many events occur between the time of photon absorption and the biologic response. The major events following photon absorption and preceding any observable effect depend on (1) the number of photons absorbed by cellular chromophores, (2) the number of excited-state molecules that undergo the "proper" photochemical reaction rather than another process, and (3) the degree of biologic response per molecule of photo-product formed.

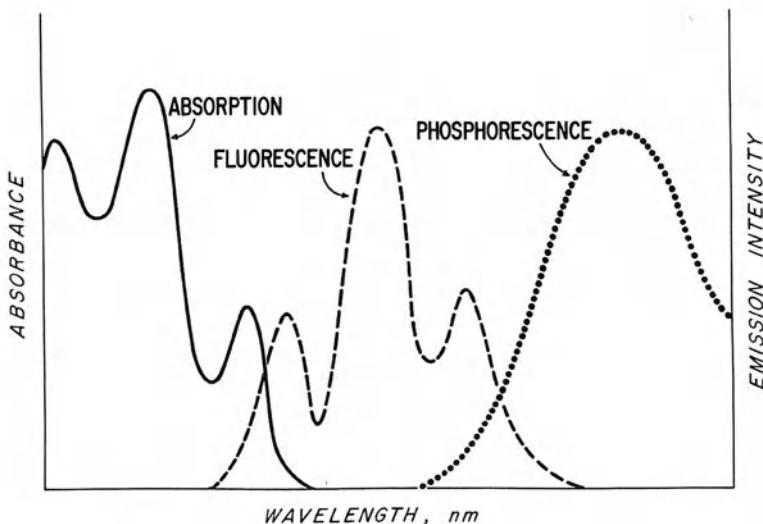


Figure 1.7. Absorption, fluorescence, and phosphorescence spectra of a molecule. The absorption and fluorescence wavelengths overlap because of transitions involving the rotation and vibration levels of the electronic states. The phosphorescence begins at longer wavelengths than the fluorescence because the triplet state has a lower energy than the singlet.

1.3.2. Types of Photochemical Reactions

For a photobiologic effect to occur, there must be photochemical reactions in the cell. When the chromophore is chemically changed during the reaction, a direct photochemical mechanism is said to be involved. The chromophore alone may be chemically changed (Equation 8a), or it may react with another molecule (Equation 8b).

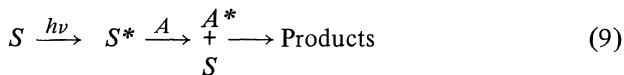
Direct mechanism



where A = molecule, A^* = excited molecule, B = another molecule, either the same as A or different, and $h\nu$ = energy of a photon absorbed by A .

When the chromophore is not changed, but causes a chemical change in another molecule, the reaction is called a photosensitized reaction (Equation 9). The chromophore is called a photosensitizer, and the mechanism can involve energy transfer or electron transfer.

Photosensitized mechanism

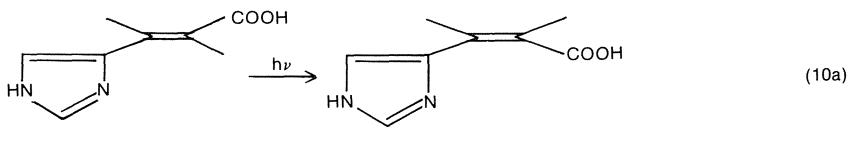


where S = photosensitizer molecule, S^* = photosensitizer molecule in excited state, A = molecule, A^* = excited molecule, and $h\nu$ = energy of a photon absorbed by S .

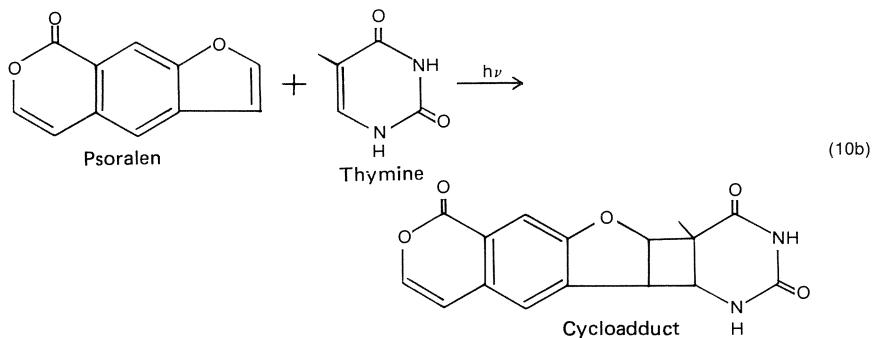
The term *photosensitizer* is troublesome. In photobiology, it is defined as a molecule that absorbs photons and causes a change in another molecule. Thus, reactions occurring by either Equation 8b or Equation 9 cause photosensitization in the photobiologic sense.

Photochemical reactions can be classified by the types of molecular changes that occur in molecular bonding. The following are examples of some basic types of photochemical reactions that occur in molecules involved in photobiology:

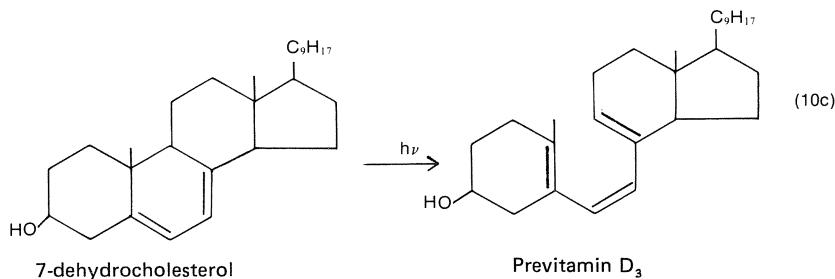
Cis-trans, isomerization around a double bond



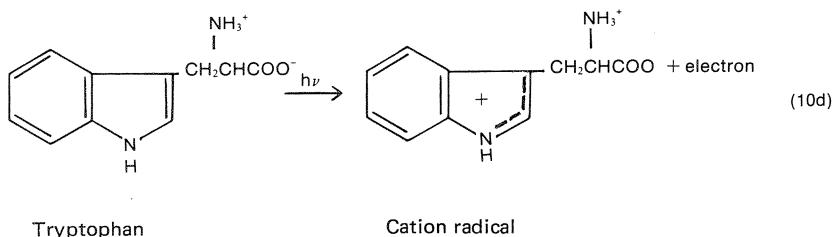
Cycloaddition



Intramolecular rearrangement



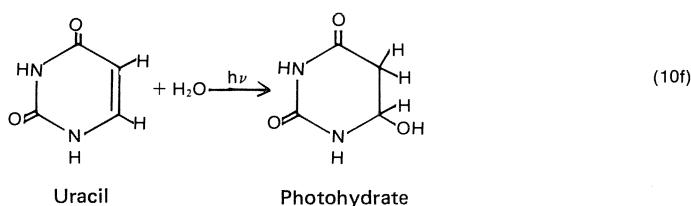
Photoionization



Fragmentation



Linear photoaddition



1.4. The Relationship between Photoproducts and Biochemical and Cellular Changes

A major goal of photobiology is learning details of the pathway between light absorption and the biologic response (Fig. 1.1), i.e., which photoproducts, enzymes, metabolites, structural proteins, or other factors are involved and how they are related. Usually, only a portion of the pathway is known, even for a common nonimmunologic human photobiologic response such as UV-radiation-induced inflammation in skin (sunburn). The chromophore for this response has not been identified, although many action spectrum determinations have been reported⁷ and much biochemical information has been generated. Photoproducts detected in skin after its exposure to UVB radiation include pyrimidine dimers in DNA, free radicals, and oxidized lipids. Biochemical responses detected in skin include time-dependent changes in DNA, RNA, and protein synthesis; increases in enzyme activities; disruption of lysosomes; and generation of mediators of inflammation. Despite such observations, the causal pathway(s) between light absorption and UVB-radiation-induced inflammation has not been identified.

The chromophores for photoimmunologic responses and their photochemistry are not known except when the chromophore is added to the system. Irradiation of lymphocytes with UV radiation elicits DNA repair processes and, with sufficient doses, causes cell death. Extensive research in other cells suggests that these responses are caused by light absorption by DNA. In addition, exposure to UV radiation inhibits the response of lymphocytes to phytohemag-

glutinin (PHA) stimulation. It has not been determined whether DNA or another cell component is the chromophore for this response, which requires competent cell membrane and several intracellular functions. Similarly, UVB-radiation-induced loss of cell surface markers from Langerhans cells may be a direct membrane effect (with the chromophores presumably being membrane proteins) or may be initiated by DNA photochemistry or by some other mechanism. The UV-radiation-induced inhibition of contact sensitivity and tumor rejection may be mediated by effects of UV radiation on Langerhans cells or by another mechanism. Solar urticaria may involve an immunologic response in some cases. Since the effective wavelengths vary among patients, it is difficult to ascribe this response to a single chromophore.

In some cases, the chromophore for a photobiologic response is known either because it has been isolated from the biologic system or because it was added intentionally or unintentionally. Studies on the chromophore are performed *in vitro* to determine the mechanisms of its photochemistry and its photoproducts. The problem then is to relate these *in vitro* results to the *in vivo* response. The criteria for a causal relationship between a photoproduct and a biologic response have been delineated.⁸ One well-studied photobiologic response is vision; the chromophore, its *in vitro* and *in vivo* photoproducts, and the subsequent biochemical steps are moderately well understood.

The photochemistry and photobiology of UV-radiation-induced cell death and mutation are also well studied. In this case, the chromophore, DNA, was identified from action spectral data and confirmed by subsequent investigations. The major photoproducts related to these responses are cyclobutyl-pyrimidine dimers. These dimers are repaired in the dark within mammalian skin cells, including those maintained in tissue culture and those studied *in vivo*. The steps in the repair that excises this lesion have been studied biochemically.⁹ Repair has also been shown to occur in these systems via an enzymatic pathway that requires visible light (photoreactivating enzyme). The detailed steps from the initial photoproducts to lethality remain to be defined in detail. In addition, other DNA photoproducts are formed (e.g., hydrates and crosslinks with protein) that may also contribute to the biologic response.¹⁰

Another human photobiologic response with a known chromophore is the cutaneous sunlight sensitivity associated with patients with porphyria. Porphyrins, which are produced in higher than normal amounts in these patients because of metabolic defects, are the chromophores for the response. *In vitro*, porphyrins photosensitize oxidation of amino acids and lipids and photosensitize membrane protein crosslinking. The relative roles of these photoproducts in the cutaneous responses are being studied currently.

The chromophore for a photobiologic response may also be of exogenous origin. Phototoxicity and photoallergy are cutaneous photosensitive responses to an added chemical. The photochemistry of many of these compounds has

been studied in vitro. Reactions such as photoaddition to DNA and protein, photosensitized oxidation of amino acids, lipids, and DNA, and formation of toxic photoproducts have been observed. The current challenge in this area is relating these observations to in vivo effects. Phototherapy of diseases such as psoriasis presumably make use of the phototoxicity of compounds such as 8-methoxypsoralen. In this case, light-induced effects on cells are beneficial to the total organism.

Photoimmunologic responses that involve the addition of known chromophores include photoallergy to various compounds and the effects of 8-methoxypsoralen plus UVA radiation on mitogen stimulation of lymphocytes and on inhibition of contact allergy. The photochemistry that results in the binding of photoallergens to proteins is discussed in Chapter 12. The effect on biologic systems of 8-methoxypsoralen plus UVA radiation is generally assumed to result from light-initiated covalent bonding of this molecule to DNA. However, this point has not been absolutely established because psoralens can participate in other types of photochemistry. In any case, the steps between the formation of photoproducts and the decrease in mitogen stimulation of lymphocytes have not been established.

In summary, we are very far from knowing the detailed relationship between photoproducts and biologic responses in most systems, including the photoimmunologic responses discussed in this book.

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Chapter 2

Photosensitization in Mammalian Cells

John D. Spikes

2.1. Introduction

In general, the photobiologic responses of cells result from chemical changes produced by photons of electromagnetic radiation absorbed by certain molecules making up the cells. In some cases, the response results from an alteration of the molecule that absorbs the photons, for example, the inactivation of an enzyme molecule in a cell after the absorption of UVC photons. In contrast, many kinds of photobiologic responses involve the alteration of one type of molecule in the cell as a result of the absorption of photons by another type of molecule. Such phenomena, which occur by way of a number of different reaction mechanisms, are termed photosensitized reactions, and the molecule that absorbs the light is referred to as the photosensitizer (or sensitizer).¹⁻⁸ Most types of cells are relatively insensitive to light and UVA radiation because the bulk of the molecules in cells do not absorb these wavelengths strongly. However, in the presence of an appropriate sensitizer that absorbs in this range, cells can be injured and killed when illuminated. This phenomenon was discovered at the end of the last century by Raab,¹ who found that paramecia were rapidly killed when exposed to light in the presence of certain acridines that, at the low concentrations used, had no deleterious effects on the cells in the dark.

The general term for the damage to or killing of cells by photosensitization is phototoxicity (when allergic responses are not involved)⁵; biologists often use the term photodynamic killing when oxygen is required for the process.¹ Since Raab's early studies, the effects of photosensitization have been examined in many kinds of cells, including prokaryotes, such as bacteria and blue-green algae, eukaryotic algae, fungi, protozoa, and cells from multicellular plants and animals, including man.^{7,8} The study of photosensitization is fundamental to our understanding of many photobiologic events, and experimental techniques using photosensitization allow us to manipulate biologic systems by the appropriate selection of sensitizers and light. In complex systems (such as humans), the site,

fundamental nature, and action spectrum of a photobiologic response can be altered and potentially made more specific by the use of photosensitizers.

This chapter will discuss the major photochemical and chemical processes involved in cellular photosensitization, the kinds of molecules that act as sensitizers and that serve as substrates (the biomolecules altered by photosensitized reactions) in mammalian cells, and the kinds of changes that occur in cell models and in cells because of photodynamic treatment. The coverage will not be encyclopedic. Rather, selected examples will be used to illustrate certain points. Reference will generally be made only to reviews and to very recent papers; these may be consulted for further information. Blum's book¹ is an excellent reference to earlier studies in the field.

2.2. Fundamental Aspects of Photosensitized Reactions at the Biomolecular Level

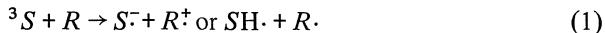
2.2.1. Introduction

Early in this century it was found that diastase, invertase, and papain could be inactivated when illuminated in the presence of photosensitizers (often termed dyes) and molecular oxygen.¹ Since then it has been shown that many different types of biomolecules can be degraded by photosensitization processes. In cells, such an alteration of essential molecules can lead to the loss or alteration of the function of particular organelles or other subcellular structures; this, in turn, can injure or kill the cell. In animals, this cellular damage or killing can lead to severe injury or even to death of the organism.^{1,8} Thus the scope of the study of the processes of photosensitization in mammals ranges from simple biomolecules, such as amino acids and pyrimidines, to man.

2.2.2. Mechanisms Involved in Photosensitized Reactions in Biologic Systems

When a sensitizer molecule, S , in the unexcited (ground) state absorbs a photon of light, it is typically converted into an energy-rich, electronically excited form termed the first excited singlet state, 1S . Very few photosensitized reactions directly involve this state because of its very short lifetime ($\sim 1-100$ ns). Good photosensitizers undergo intersystem crossing from the excited singlet state to give high yields of the triplet state, 3S ; this form of the molecule, because of its longer lifetime (~ 1 ms to 1 sec), can mediate photosensitized reactions with high efficiency.^{2,4}

Photosensitization can proceed by many different reaction mechanisms.²⁻⁸ Most of those that have been analyzed require molecular oxygen; however, there are some exceptions. In type I reactions, free radical or direct reactions, the sensitizer molecule reacts directly with a (typical reducing) biomolecule, R , by electron (or hydrogen atom) transfer to give a semireduced (free radical) form of the sensitizer, $S\cdot$, and a semioxidized (free radical) form of the biomolecule, $R\cdot$, as follows:



Less frequently, sensitizer molecules in the triplet state can donate an electron to molecular oxygen with the formation of superoxide, O_2^- . The free radicals formed in reaction 1 can react further, especially in the presence of oxygen, in numerous ways to give a variety of reaction products, often ground-state sensitizer and an oxidized form of the substrate. Some semireduced sensitizers can react as follows to give hydrogen peroxide:



Further, some semireduced sensitizers react efficiently with oxygen to give ground state sensitizer and superoxide:



In other cases, sensitizer and substrate radicals interact to produce covalent sensitizer-substrate and substrate-substrate photoadducts:



The formation of noncovalent ground state sensitizer-substrate complexes, before illumination, enhances the probability of the photochemical formation of sensitizer-substrate photoadducts because of the proximity of the excited sensitizer to the functional groups on the substrate molecule.^{2,6,8,9}

In type II reactions, (singlet oxygen or indirect reactions), triplet sensitizer reacts with ground state molecular oxygen, 3O_2 , by an energy transfer process to give a ground state sensitizer and a singlet excited state of oxygen, 1O_2 :



This form of oxygen is much more electrophilic than ground-state oxygen and typically reacts very rapidly with electron-rich regions of biomolecules to give oxidized forms of the molecules.^{2,5,8}

Many kinds of naturally occurring and synthetic organic, metallo-organic, and inorganic compounds act as photosensitizers, including acridines, bilirubin, flavins, haloaromatics, ketones, phenothiazines (such as chlorpromazine and methylene blue), polycyclic hydrocarbons (such as hypericin), polyenes, porphyrins that do not contain transition-state elements such as iron, a number of psoralens (furocoumarins), pyridoxal, xanthene dyes (rose bengal and eosin), ruthenium bipyridyl, cadmium sulfide, and Fe³⁺ and Cu²⁺ ions.^{1,2,7,8} Some of these sensitizers are endogenous (occur naturally in cells); others are exogenous (come from the environment). Some sensitizers are natural compounds (certain polycyclic hydrocarbons, furocoumarins, and chlorophylls in plants), but most are synthetic. Most of the compounds listed above function as photosensitizers only in the presence of molecular oxygen. However a few (e.g., psoralens and chlorpromazine) can function efficiently in the absence of oxygen.

In summary, sensitized photoreactions may involve photoexcited states of molecules, free radicals, and/or reactive oxygen species, including singlet oxygen, hydrogen peroxide, superoxide, and possibly the hydroxyl radical.^{10,11} The overall chemistry of these photoprocesses often appears to be simple; however, the reactions can be very complex, and the intermediate steps involved, in general, are not well understood. The study of photosensitization phenomena has expanded in recent years, primarily because of the increased use of photosensitized reactions in medicine, in particular, the use of psoralens as sensitizers in the phototreatment of psoriasis and the use of porphyrins as targeted sensitizers in the phototreatment of tumors.⁸

2.2.3. The Alteration of Biomolecules in Vitro by Photosensitization

The effects of photosensitization on most of the major types of cell molecules in solution have been examined.^{2,4,8} Most such molecules are fairly rapidly photodegraded under aerobic conditions by type I and/or type II reactions. For example, proteins are altered by attack at one or more of the following residues: Cys (oxidized to cystine, or less commonly, to cysteic acid), His (imidazole ring destroyed), Met (converted to methionine sulfoxide and/or methional), Trp (converted to kynurenine derivatives), and Tyr (ring rupture giving unknown final products). Such reactions can lead to loss of enzyme or hormone activity, changes in conformation, molecular aggregation, and changes in the mechanical properties of structural proteins.^{2,8,12,13} Oxidation induced by photosensitization progressively destroys the ability of a protein to react toward an anti-serum; however, some enzymes can be inactivated without changing their reactivity toward antisera to any great extent. Similarly, photodynamic treatment of some proteins progressively reduces their antigenicity, although carefully controlled photooxidation can destroy the toxicity of certain bacterial toxins with-

out appreciably changing their antigenicity. The oxidation of antibodies induced by photosensitization ultimately destroys their reactivity; however, a moderate treatment can convert them into nonagglutinating and nonprecipitating forms that still react specifically with their antigens.¹³⁻¹⁵

Other alterations of biomolecules *in vitro* induced by photosensitization include effects on unsaturated lipids, including cell membrane lipids, which are typically oxygenated at double bonds to give mixtures of allylic hydroperoxides. Steroids, such as cholesterol, are also converted to hydroperoxides. Nucleic acids are somewhat selectively attacked at the guanines in a reaction that destroys the purine ring structure, and single-strand breaks sometimes result. Carbohydrates are slowly attacked at the hydroxyl groups by a type I process that produces products such as hexonic acids and ketones. Other minor compounds including ascorbic acid, folic acid, biotin, tocopherols, and lipoic acids are also photooxidized.^{2,3,4,8,11,12}

The kinetics, reaction mechanisms, and final products of the photosensitization of biomolecules described above have been studied using many different sensitizers and a wide range of reaction conditions. A number of mechanistic tests have been devised to establish the relative participation of type I and II processes in these reactions, but none give unambiguous results.^{2,3,4,8,9} One approach is to examine the effects on photooxidation rates of inhibitors that do not react with the various excited states of the sensitizer, but which quench singlet oxygen with high efficiency. The participation of singlet oxygen in the process is suggested when the rate is inhibited at low concentrations of azide or of other inhibitors of this type. Singlet oxygen has a much longer lifetime in D₂O than in H₂O. Thus a reaction mediated by singlet oxygen would be expected to go much faster in D₂O. A second approach is to examine the chemical nature of the photooxidation product(s) to identify the reaction mechanism involved. A third approach is to use flash photolysis studies.⁸ However, although extensively studied, the details of the mechanisms by which most biomolecules are photooxidized are not known.

Other, less common, kinds of photoalteration reactions are observed with certain sensitizer-substrate combinations. For example, in solution, light-excited psoralens (furocoumarins) add across the 5,6-C=C bond of pyrimidine bases to give covalent furocoumaryl-pyrimidine cycloadducts; oxygen is not required for and can actually inhibit, this process.¹⁶⁻¹⁸ This represents a photosensitization process in the sense that the pyrimidine undergoes a chemical change as a result of radiation absorbed by the psoralen molecule. Many psoralens intercalate between two base pairs in double-stranded deoxyribonucleic acid (DNA) and in ribonucleic acid (RNA) in solution. Subsequent illumination of these complexes results in the formation of covalent adducts between psoralen molecules and bases. Depending on the chemical structure of the psoralen and the illumination technique used, these can be monoadducts (psoralen-base) or diadducts (base-psoralen-base); in the latter case, cross-links are formed in the nucleic acid molecule.^{5,16-18} Chlorpromazine, a phototoxic drug used in the therapy of

psychiatric patients, forms covalent photoadducts with proteins and with DNA when irradiated.⁶

Many typical aerobic photodynamic sensitizers also couple with biomolecules when illuminated with visible light (e.g., fluorescein couples to bovine serum albumin, RNA, and dextran, and methylene blue couples to ribonuclease). Coupling also occurs in these systems in the absence of oxygen, but with much lower yields.¹⁹ When a solution containing fluorescein, Trp, and fibrinogen is illuminated, the amino acid couples covalently to the protein, mainly at the regions of the fibrinogen exposed to the solvent.²⁰ This type of reaction can be used to study conformational changes in proteins in solution. The formation of covalent intermolecular cross-links between protein molecules of the same kind in solution also has been observed. For example, illumination of aerobic solutions containing human hemoglobin and methylene blue gives good yields of covalent hemoglobin-hemoglobin dimers and traces of higher molecular weight products. The rate of cross-linking is decreased by azide and enhanced in D₂O, suggesting that singlet oxygen is involved in the reaction.²¹ Similar results have been observed with other proteins and sensitizers.²² Little is known about the mechanism of these cross-linking reactions. Bilirubin forms covalent photoadducts with serum albumin during the standard phototherapy of hyperbilirubinemic infants; the photobinding appears to occur at a specific region of the serum albumin molecule, probably at the binding site for bilirubin.²³

When DNA in solution is exposed to UVC radiation, covalent cyclobutane-type pyrimidine (mainly thymine-thymine) dimers form in the nucleic acid. Added photosensitizers, such as acetone and ethyl acetate, can sensitize this reaction to wavelengths of light absorbed by the added material. The energy transfer involved in the reaction does not occur by a simple physical mechanism, but apparently depends on the ability of the excited sensitizer molecule to form a complex with a pyrimidine.²⁴ UVC irradiation causes the formation of covalent complexes between DNA in solution and several other cellular components including most amino acids and numerous different proteins.²⁵ The cross-linking of nucleic acids with proteins in solution can also be produced with light in the presence of appropriate photosensitizers such as acridine orange.²⁶ Many of these same kinds of crosslinking processes occur *in vivo* and are probably involved in the injury and killing of mammalian cells associated with photosensitization.

2.3. Sensitized Photoreactions in Model Cell Systems

2.3.1. Introduction

Like the photosensitized reactions in homogenous aqueous systems already discussed, the photosensitized reactions that result in injury or killing of mam-

malian cells are complex processes. Cells are not homogeneous; they contain many microenvironments with different physicochemical properties ranging from hydrophilic to lipophilic. Furthermore, because of these localized chemical differences, most sensitizers, depending on their properties, selectively dissolve in or bind noncovalently to particular microregions of the cell. Because of these complexities, we know relatively little about the details of photosensitized reactions in cells or of the specific lesions leading to cell injury and death. In biology, a preliminary, but sometimes useful, approach to the study of such complexity is to work with so-called model systems that simulate, albeit in a grossly oversimplified manner, certain properties of the living cell. Three models that have been used to study these reactions will be discussed here: photosensitizers bound to macromolecules, photosensitizers in aqueous detergent micellar systems, and photosensitizers in liposome systems.

2.3.2. Sensitizers Bound to Biologic Macromolecules

Many sensitizers form noncovalent complexes, sometimes selectively, with biologic macromolecules, such as proteins and nucleic acids. Thus, photochemical studies of such systems *in vitro* provide some indication as to the probable behavior of these sensitizers when they are bound to the same kinds of molecules in cells. The binding of sensitizers to biopolymers can affect the mechanism of the photosensitized reaction in that type I processes will be enhanced because of the proximity of the sensitizer molecule to reactive groups on the substrate. Furthermore, the efficiency of the oxidation of proteins, and especially of nucleic acids, is often increased by sensitizer binding. Also, in some cases, bound sensitizers show an increased yield of triplet formation when exposed to light.^{5,9} Illumination of proteins in the presence of exogenous sensitizers bound to specific sites on the molecule results in the alteration of only those sensitive amino acid residues located very near the bound sensitizer. Some proteins contain endogenous photosensitizing moieties located at specific sites; illumination of such molecules (for example, the pyridoxal-dependent dehydrogenases) results in the destruction of amino acids located near the cofactor binding site. Thus, such photosensitized reactions can be very selective and, in some cases, may be used to probe the three-dimensional structure of macromolecules.^{8,27}

2.3.3. Aqueous Micellar Systems

Aqueous preparations of amphipathic surfactants above their critical micellar concentrations are sometimes used as models of cell membranes because the aggregates formed have both hydrophilic and hydrophobic regions. Both lipid-soluble substrates and sensitizers are readily solubilized in micelles in aqueous suspension. Recent studies of photosensitized reactions in micellar

systems^{5,9,28} have added considerably to our understanding of these systems. Typical micelles, prepared from sodium dodecyl sulfate in an aqueous medium, for example, are roughly spherical dynamic aggregates of approximately 100 monomers. These aggregates are organized with the hydrocarbon tails of the molecules located in the interior (thus creating a hydrophobic environment) and with the polar heads of the molecules on the exterior forming an outer shell in contact with the solvent. Various combinations of sensitizers and substrates in the same and in different micelles and dissolved in the medium have been examined in photosensitization studies. Although the results are somewhat complex, they do show that singlet oxygen is generated with high efficiency by the illumination of sensitizers located inside micelles; it can then diffuse across phase boundaries and out of the micelle where it can oxidize external substrates; it can also diffuse into the interiors of micelles and react with substrates located there. Electrons photogenerated by sensitizers located inside the micelles can also diffuse out of the micelles and through the medium and then attack substrates located in other micelles. Micellar charges can have a great effect on such processes. Attempts are being made to apply the results of these kinds of studies to an understanding of photosensitization processes occurring in cells.^{9,28}

2.3.4. Liposome Systems

Liposomes (microscopic bilayered phospholipid vesicles) have been used extensively as model systems for studying certain aspects of mammalian cell membrane behavior.²⁹ Lipid-soluble photosensitizers and substrates can be incorporated into the liposomal membrane during preparation, and water-soluble sensitizers and substrates can be entrapped within the liposome or added externally. The membranes of liposomes prepared from unsaturated phospholipids, such as egg phosphatidylcholine (egg lecithin), are attacked by singlet oxygen and superoxide which are generated by the illumination of water-soluble photosensitizers in the medium; this results in an increased permeability and ultimately in lysis of the membrane as a result of peroxidation of the phospholipid.^{30,31} When α -tocopherol or β -carotene, which protect against photodynamic attack, are incorporated into the liposome membrane, both peroxidation of the lipid and lysis of the membrane are inhibited; free-radical quenchers can also protect the membrane.^{30,32} Liposomes prepared from saturated phospholipids, such as dipalmitoylphosphatidylcholine, are not lysed by photodynamic treatment.³¹

When hematoporphyrin is incorporated into liposomal membranes containing cholesterol, the singlet oxygen photooxidation product of cholesterol is produced when the liposomes are illuminated; external hematoporphyrin in the surrounding aqueous medium is much less effective.³³ Lipid-soluble porphyrins, such as hematoporphyrin dimethyl and dipalmitoyl esters, incorporated into the membranes of liposomes prepared from saturated phospholipids, sensitize the

photooxidation of external substrates, such as amino acids and proteins with quantum yields similar to those observed with porphyrins in aqueous solution.³⁴ These photochemical studies using liposomes are in an early stage; as additional work is done, information obtained from this model system should improve our understanding of photosensitization processes in the membranes of mammalian cells.

2.4. Photosensitization Processes in Mammalian Cells

2.4.1. Introduction

It was shown early in this century that rabbit erythrocytes treated with porphyrin photosensitizers hemolyzed when illuminated.¹ Since then, mammalian cells have been used extensively in photosensitization studies. Much of this work has used porphyrins as sensitizers because of the photosensitivity observed in certain human porphyrias³⁵ and, more recently, because of the use of porphyrins as sensitizers in the phototherapy of animal and human tumors.³⁶

We can predict to some extent the kinds of lesions that will be produced in photosensitized cells from the information presented in the previous sections.^{9,37} For example, cell membranes contain unsaturated lipids, structural proteins, and enzymes, all of which can be degraded by photosensitization. Thus, if the sensitizer is localized in the membrane, illumination would cause changes in membrane permeability, mechanical properties, and alterations in catalytic and active transport processes. These changes would seriously interfere with cell metabolism and also lead to membrane disruption and cell lysis. Sensitizers would also localize in many regions of the cytoplasm and thus mediate the photodegradation of enzymes, tRNA, and components of cell organelles. Finally, if the sensitizer penetrated into the nucleus, illumination would cause the destruction of guanines in DNA, strand breakage, and the formation of DNA-protein photoadducts; these alterations, in turn, would interfere with cell division and give rise to mutations. As we shall see in this section, all of these predicted changes do occur in photosensitized cells. Most of the reactions that have been studied require molecular oxygen, but a few categories, depending on the sensitizer, do not.

2.4.2. Effects Mediated by Culture Medium Components and by Endogenous Sensitizers

Some precautions must be taken in performing photosensitization experiments with mammalian cells because photodamage can result not only from added sensitizers, but also from components of the culture medium and from

endogenous photosensitizing components in the cells. For example, many kinds of mammalian cells are injured or killed by exposure to daylight or cool white fluorescent lamps (which emit light and UVA radiation) in the presence of many kinds of cell culture media. Studies have shown that cell killing, the formation of cross-links and breaks in cellular DNA, sister chromatid exchanges, or accelerated neoplastic transformation can occur. In some cases, at least, these effects are mediated by hydrogen peroxide produced photochemically by riboflavin and Trp in the culture medium.³⁸⁻⁴⁰ Many of these same kinds of effects are observed with some types of cells when they are illuminated in balanced salt solutions or in culture media lacking riboflavin and/or Trp. In these cases, the damage is probably mediated by light absorbed by the flavin and/or heme moieties of certain enzymes. In some cases, the damage is rather selective. For example, the illumination of isolated rat hepatocytes in a flavin-free medium has little effect on the cell membranes but severely damages mitochondria, microsomes, and catalase; isolated mitochondria and microsomes are also damaged by light.⁴¹⁻⁴³ The action spectra for both medium- and endogenous-sensitizing phenomena peak in the long-wavelength UV and blue regions of the spectrum. Thus, as a general precaution, cell culture and media preparation and storage areas should be illuminated with light sources emitting only at longer wavelengths.

2.4.3. Sensitizer Penetration and Subcellular Localization

The sensitizer and substrate must be located quite close together for efficient sensitized photooxidation to occur. Type I reactions appear to act only over rather short distances. In type II processes, the singlet oxygen produced, because of its short lifetime, cannot diffuse for more than approximately 100 nm through an aqueous medium⁷; in cellular systems in which many reactive substances are present, it probably does not diffuse even this far. Thus, for the effective photosensitization of cells, the sensitizer must penetrate into the cell and end up in close association with the subcellular structure involved. A major goal in photosensitization studies at present is to determine the mechanism(s) of sensitizer movement into cells as well as the subsequent localization of the sensitizer. Only when these patterns are clearly established can we hope to describe these photodynamic effects in detail. The pattern of localization depends on a number of factors, including the physicochemical properties of the sensitizer molecule (molecular size, charge, water/lipid partition coefficient), the type and physiologic state of the cell and the environmental conditions. Sensitizer molecules presumably diffuse into the cell membrane, or through it and on to other sites. The possible involvement of active transport processes has not yet been studied. Many types of mammalian cells in culture take in materials by endocytosis and phagocytosis; however, whether these processes participate in the penetration of sensitizer has not been established. It has been

suggested that the uptake of various dyes by lysosomes in cells may reflect the pinocytotic activity of the cell.⁴⁴

Several techniques can be used to trace the movement of sensitizers into cells. Fluorescence microscopy is often used because almost all photosensitizers are fluorescent. This technique was used in early studies of several types of cells. The studies showed that (1) eosin and rose bengal do not penetrate through the cell membrane (in fact, eosin penetration is used as an indicator of cell death), (2) uroporphyrin I, neutral red, and acridines accumulate in lysosomes, and (3) acridines also accumulate in the nucleus.⁴⁵ Fluorescence microscope studies with meso-tetra(*p*-sulfophenyl)porphine showed that the sensitizer is evenly distributed in the cytoplasm of Vero cells, whereas it is associated with various cell organelles, especially the nucleolus, in HEp-2 cells.⁴⁶ Liposomes loaded with hematoporphyrin dimethyl ester appear to accumulate almost quantitatively in the lysosomes of HeLa cells.³⁴

Penetration of sensitizer can also be determined by measuring the depletion of sensitizer from the medium and/or its accumulation in cells by chemical or photometric assays. Using such techniques, investigators have found that lipid-soluble and highly water-soluble porphyrins are poor sensitizers for cells. The most effective compounds have octanol to phosphate-buffered saline partition coefficients in the range of 2-12.⁴⁷ Such porphyrins would be expected to localize at hydrophobic-hydrophilic interfaces in cell membranes rather than in highly hydrophilic regions of the cell or in the highly hydrophobic core of membranes. Similar results have been found with a series of xanthene sensitizers and nonmammalian nerve cells.⁷ Human cells (NHIK 3025) take up the sensitizer hematoporphyrin more slowly at 4°C than at 37°C; uptake is enhanced by lowering the pH of the medium and by illumination but is decreased in the presence of serum, which is known to bind many porphyrins.⁴⁸ When cells are incubated in solutions of porphyrins, the sensitizer initially moves into the cells very rapidly, and then, after a time, more slowly. Porphyrin can be washed out of the cells much faster after a short incubation period than after a long one. The initial rapid accumulation of the sensitizer may occur because the sensitizer binds to the more hydrophilic regions of the cell; the sensitizer would wash out easily from these sites. Presumably, the slowly accumulating porphyrin localizes in more hydrophobic regions of the cell and, therefore, would wash out less rapidly.^{48,49}

Another technique for determining the subcellular localization of sensitizers is to disintegrate the treated cells and then separate out the various subcellular components; the sensitizer concentration in each fraction can then be determined. Studies of V79 Chinese hamster lung fibroblasts treated with hematoporphyrin derivative showed that most of the sensitizer was in the soluble cytoplasmic protein fraction, approximately 19% was in the microsomal fraction, 14% was in the mitochondrial fraction, and only about 4% was in the nuclear fraction.⁵⁰

2.4.4. The Kinetics of Cell Killing

Cell death is often used as an end point in assessing the effects of photosensitization treatment. Because of its complex nature, investigators have defined cell death in various ways.⁷ Perhaps the most commonly used technique is to culture the treated cells and then determine the fractions that do not divide and produce colonies. This identifies cells that have undergone what may be termed reproductive death; such cells, however, can often still carry out a number of normal metabolic functions. With many types of cells, reproducible criteria for cell death can be established by observing cell morphology with a phase-contrast microscope.³⁴ Another approach is based on the functional state of the cell membrane. Live cells, by this criterion, exclude certain kinds of molecules, such as trypan blue; dead cells are rapidly stained by the dye.

Data from studies of sensitized phototoxicity are traditionally presented by plotting the logarithm of the fraction of cells surviving on the vertical axis of a graph vs. the duration of light exposure (light dose) on the horizontal axis. The resulting plots typically have an initial curved region (shoulder), followed by a linear region corresponding to the exponential photokilling of the cells. The presence of the shoulder region indicates that the cell has some ability to repair sublethal photodamage occurring in the low-dose region or that a multiple-hit photochemical process is leading to cell death. The width of the shoulder and the slope of the exponential region of the plot depend on the cell type, the physiologic conditions of the cells, the sensitizer, and the experimental conditions. Several cell lines, as studied in synchronized cultures, show a marked difference in photodynamic sensitivity at different stages of the cell division cycle. The sensitivity is typically lowest in early G₁, late S, and G₂ phases; it increases through the late G₁ and early S phases and reaches a maximum in the middle S phase.^{51,52} The increased sensitivity in the S phase is manifested as a decrease in the width of the shoulder region of the survival curve; the slope of the exponential region does not change significantly. This suggests that the repair capabilities of the cell may be lowest in the middle S phase. However, some cell lines show no significant change in photosensitivity during the cell division cycle.⁵³

Several reaction variables affect the killing of cells by photosensitization. Increasing the sensitizer concentration typically decreases the shoulder width and increases the slope of the exponential region of the survival curve.⁵³ With hematoporphyrin-sensitized NHIK human cancer cells, the survival curve for illumination at 37°C has a distinct shoulder, whereas at 4°C, no shoulder is observed. These data indicate that the shoulder region at 37°C reflects a repair process rather than a multiple-hit type of killing.⁵⁴ Split-dose experiments, in which the cells are incubated for various times in the dark between two fixed light exposures, show that cell survival decreases with increasing dark periods.⁵⁴

The mechanism by which cells are killed by photosensitization has also been studied. For example, the photokilling of HeLa cells sensitized with toluidine blue dye is markedly decreased in the presence of histidine (a singlet oxygen trap) and enhanced in a medium containing D₂O.³⁷ Intracellular 1,3-diphenylisobenzofuran (another efficient singlet oxygen trap) protects cells against photokilling with hematoporphyrin derivative,⁵⁵ whereas media made up in D₂O enhance photosensitized killing with hematoporphyrin.⁵⁶ Cells incubated for some time in the presence of β-carotene³² are protected from photokilling with hematoporphyrin.⁵⁷ All of these results suggest a major involvement of singlet oxygen in the killing process with these sensitizers. This interpretation is further supported by the observation that singlet oxygen is produced in illuminated TA-3 mouse mammary carcinoma cells containing hematoporphyrin derivative with an efficiency of approximately 0.16 molecules per photon of light absorbed by the sensitizer. It requires the input of approximately 1.5 to 3.6×10^9 photons of 620 nm light per cell to kill 90% of TA-3 cells, depending on the intracellular concentration of hematoporphyrin derivative.⁵⁸ These results are similar to the early studies of Blum⁸ that showed that the absorption of approximately 10^{12} photons by a photosensitized erythrocyte was required for hemolysis.

In summary, considerable data on phototoxic reactions in cells have been gained from studies of the kinetics of cell killing. Killing, however, is a rather nonspecific end point for assessing a cellular response because it can be the result of many different kinds of lesions initiated by a variety of molecular level photochemical events. For this reason, much of the recent research on cell photosensitization has been directed toward a more refined elucidation of the mechanisms of different kinds of cell damage.

2.4.5. Membrane Effects

The cell membrane represents a major site of photodynamic damage, and many studies on this subject have been published.^{7,8,35} Mammalian erythrocytes have been used extensively for such studies because the mature cells lack nuclei and most of the ordinary cytoplasmic structures; thus, they represent perhaps the simplest model cell system available for studying membranes. Furthermore, the cell contents can be easily removed from erythrocytes, leaving "ghosts" that are almost pure membrane. An excellent analytic review of studies of photosensitization with red blood cells has recently appeared.⁷ One of the major areas of research has been on photohemolysis. When illuminated in the presence of sensitizer, erythrocytes swell and then rupture, releasing the cell contents. This response results from the photodynamic formation of small pores in the cell membrane that increase the passive permeability of the cell to small cations, leading, in turn, to colloid osmotic lysis. Although many different kinds of

sensitizers are effective in photohemolysis,^{7,35} most recent work has been performed with porphyrins, especially protoporphyrin IX (because of its apparent involvement in the photosensitivity of individuals with erythropoietic protoporphyrria). Oxygen is required for photohemolysis with most sensitizers; α -tocopherol, which is present in the red blood cell membrane, and β -carotene inhibit the reaction. Singlet oxygen, and perhaps free radicals, may be involved in aerobic photohemolysis, but superoxide and hydrogen peroxide are not.^{8,59}

The degradation of the membrane proteins and lipids by photosensitization and the role this degradation plays in the hemolysis process have been studied recently.⁷ One of the primary events in the photodynamic treatment of red blood cells is the photooxidation of susceptible amino acid residues in membrane proteins that make up approximately 50% of the membrane. As a result, many different membrane-associated enzymes are inactivated, including glyceraldehyde-3-phosphate dehydrogenase, magnesium- and sodium-dependent ATPases, and acetylcholinesterase. There are also profound effects on membrane function, in particular, an inhibition of the active transport of sodium and potassium ions and the carrier-mediated transport of glucose, L-leucine, sulfate, and glycerol. The nonspecific permeability of glycerol and thiourea is increased. In addition, there is an extensive covalent cross-linking of certain membrane proteins, including spectrin and bands 4.1 and 6. Typical anti-oxidants, such as butylated hydroxytoluene, do not inhibit the cross-linking process.^{7,60-66} Considerable work has been done on the biochemistry of the cross-linking process, but the results are somewhat conflicting.^{65,66}

Unsaturated lipids in the erythrocyte membrane, such as phospholipids and cholesterol, are rapidly photooxidized when treated photodynamically.^{7,35} For example, the illumination of red blood cell ghosts in the presence of protoporphyrin IX efficiently converts membrane cholesterol to its singlet oxygen photo-oxidation product; when this oxidized form of cholesterol is incorporated into normal erythrocytes, the membrane becomes increasingly fragile and then ruptures.⁶⁶ There is considerable disagreement at present as to the critical lesion(s) leading to the hemolysis of photodynamically treated red blood cells. Some investigators argue that hemolysis results from protein alteration; others feel that it is a consequence of the oxidation of membrane lipids.^{5,7,64,67,68}

Photodynamic effects on the membranes of many lines of tissue cells in culture have also been reported. Porphyrin photosensitizers, at least, appear to bind to cultured cells, thereby sensitizing the membrane to photodynamic damage. Porphyrins that do not bind because of their high water-octanol partition coefficients do not sensitize cells efficiently. Porphyrins, such as hematoporphyrin and deuteroporphyrin IX, which bind and are good sensitizers at 37°C, bind only poorly to L1210 murine leukemia cells at 0°C and are ineffective sensitizers.⁴⁷ This lack of effectiveness of external sensitizers is difficult to understand. The diffusion range of singlet oxygen should allow it to move

easily throughout the cell membrane when generated by external sensitizers. Perhaps singlet oxygen generated in the membrane is more effective in some way than that produced externally, or perhaps membrane-bound sensitizers act primarily by type I reactions.¹⁰

Since sensitizers do not penetrate into cells instantly, the length of the cell-sensitizer incubation period can be an important determinant of the sites of photodynamic injury in the cell.³⁷ For example, when fetal bovine fibroblasts are incubated with hematoporphyrin for 5 min and then illuminated, most of the immediate injury occurs in the cell membrane (bleb formation). Photosensitization is prevented when the cells are incubated with medium containing serum (which removes the membrane-bound porphyrin) before illumination. When the cells are incubated in the sensitizer for 2 hr, light exposure causes severe nuclear damage.⁵⁷

Photodynamic injury to tissue cell membranes is expressed in many different ways including bleb formation, hole formation, increased passive permeability to molecules, cross-linking of membrane proteins, and inhibition of transport processes. For example, illumination of mouse macrophages sensitized with rose bengal, which binds to, but does not penetrate through, the membrane, results in the leakage of potassium from the cells and in the formation of large blebs on the membrane.⁴⁵ When illuminated, protoporphyrin IX-sensitized human fibroblasts develop extensive surface blebs and, with increasing exposure time, show increasing permeability to trypan blue dye; increased permeability is paralleled by a decreased rate of cell protein synthesis. There is also a dramatic loss of protein from the illuminated cells. The presence of dithiothreitol, ascorbic acid, and α -tocopherol delays the development of cell damage.⁶⁹ At high light doses, the cytoplasmic enzyme, lactic dehydrogenase, rapidly leaks out of NHIK 3025 human tumor cells sensitized with hematoporphyrin derivative.⁷⁰ Membrane sulphydryl groups, which are essential for membrane integrity, are rapidly destroyed in illuminated human fibroblast cells sensitized with protoporphyrin.⁷¹ Protoporphyrin binds to the surface membrane of #745 Friend erythroleukemia cells and P-1081 myelocytic leukemia cells as shown by fluorescence microscopy. When illuminated, holes develop in the membrane as demonstrated by scanning electron microscopy, but the nuclear membrane is unaffected.⁷² Illumination of human neutrophils in the presence of protoporphyrin, which binds to the cells, rapidly inhibits locomotion; uroporphyrin, which does not bind, is inactive as a sensitizer. Potassium ions and lactic dehydrogenase leak out of the illuminated cells. The photodynamic effect may act directly on motility, or it may operate by activating complement.^{73,74} Photodynamic treatment inhibits phagocytosis in polymorphonuclear leukocytes.⁷⁵

As with red blood cells, the photodynamic treatment of tissue cells causes cross-linking of membrane proteins. For example, the illumination of L1210 murine leukemia and SS-1 ascites tumor cells in the presence of appropriate

sensitizers causes a substantial cross-linking of membrane proteins as measured by analytic sodium dodecylsulfate-polyacrylamide gel electrophoresis; no cross-linking of membrane glycoproteins is observed. The degree of membrane hydrophobicity of these two lines of cells decreases as measured by partition techniques.⁷⁶ The porphyrin-sensitized photodynamic treatment of L1210 murine leukemia cells also inhibits the transport of uridine and cycloleucine into the cells; sugar transport is not affected. Actinomycin D moves into the cells more rapidly after treatment because the barrier that protects untreated cells from this drug is disrupted.⁴⁷ With low-intensity illumination, rat mast cells sensitized with protoporphyrin permanently lose their ability to secrete histamine after drug stimulation.⁷⁷ Whether these effects on mast cell response are involved in photoimmunological reactions is not known.

An interesting new technique using photochemical sensitizers has recently been proposed for labeling certain kinds of proteins in cell membranes.⁷⁸ In general, only the residues of proteins exposed to solvents are susceptible to sensitized photooxidation. When intact red blood cells are illuminated in the presence of a photodynamic sensitizer and tritiated Trp, the Trp binds to the cells. Analysis using polyacrylamide gel electrophoresis shows that only those proteins known to be located at the membrane surface are labeled with radioactive Trp (band 3 protein and the major sialoglycoproteins). When lysed erythrocytes are treated similarly, all of the major membrane proteins become labeled. This technique may be useful for the specific labeling of surface molecules in biologic membranes.

2.4.6. Cytoplasmic Effects

Some sensitizers move through the cell membrane and localize in various cytoplasmic sites³⁷; subsequent illumination then damages cytoplasmic molecules and organelles. The illumination of monkey kidney cells and mouse macrophages in culture after treatment with anthracene or uroporphyrin I, which accumulate in the lysosomes, increases the permeability of lysosomal membranes as monitored by the penetration of β -glycerophosphate into the organelles.⁴⁵ On the basis of this and other studies, investigators have suggested that the rupture of the lysosomes is a primary pathway in the photodynamic injury of cells with lysosome-entrapped sensitizers. The rupture releases hyrolytic enzymes that, in turn, cause cell injury and death. More recent studies, however, indicate that lysosomal damage may be a rather late event following photodynamic treatment and thus may not be an important factor in photodynamic injury.^{70,73,74,79}

In C3HST4 lymphoma mouse cells sensitized with hematoporphyrin, the first ultrastructural change after exposure to light as observed by electron microscopy is in the mitochondria. These organelles become contracted or swollen with ruptured cristae; after longer periods, they are not recognizable. The vesicles of the rough endoplasmic reticulum also became dilated and swol-

len, whereas the ribosomes and Golgi apparatus remain essentially normal.⁸⁰ Irradiation of human neutrophils in the presence of protoporphyrin (but not uroporphyrin) inactivates cytoplasmic enzymes in this order: (1) succinic dehydrogenase (membrane-bound to mitochondria), (2) cytosol lactic dehydrogenase, (3) soluble mitochondrial glutamic dehydrogenase, and (4) lysosomal acid phosphatase.^{73,74} These data plus observations of cellular fine structure suggest that, with at least some sensitizer-cell combinations, the mitochondria are the first site of photodynamic attack in the cytoplasm. It may be that protoporphyrin somewhat specifically sensitizes mitochondria because it is lipid soluble and therefore tends to localize in the lipid regions of these organelles. Intracellular nucleoside kinases are markedly inhibited in photodynamically treated L1210 leukemia cells, and synthesis of both RNA and protein is sharply decreased; the latter effects may result from the inhibition of precursor transport into the cells.⁴⁷ RNA synthesis is also inhibited in several other cell lines.⁷²

A powerful approach to elucidating the mechanisms involved in the photodynamic damage of cell organelles is to isolate them from cells for study in a purified form. Using this approach, investigators found that liver lysosomes treated with acridine orange, eosin Y, and light release substantial amounts of hydrolytic enzymes.⁸¹ Protoporphyrin also sensitizes enzyme release from isolated lysosomes, but only after prolonged illumination.⁸² The photodynamic damage of isolated mitochondria has been extensively studied. Many effects, depending on the sensitizer type and concentration as well as the time of incubation and illumination, have been described. These include swelling, disruption of the mitochondrial structure, inhibition of respiration, uncoupling and inhibition of oxidative phosphorylation, modification of inner membrane proteins, inactivation of enzymes, and possible cross-linking of components.⁸²⁻⁸⁵ Isolated mitochondria are more sensitive to photodynamic treatment with protoporphyrin than with isolated lysosomes.⁸² Photodynamic treatment of isolated rat liver microsomes with methylene blue dye inactivates the mixed function oxidase system and peroxidizes microsomal lipids; studies with D₂O and singlet oxygen traps suggest that these reactions are mediated by singlet oxygen.⁸⁶

2.4.7. Nuclear Effects

Some sensitizers penetrate through the cytoplasm and into the nuclei of cells where they damage the nuclei when subsequently illuminated.³⁷ Sensitizers located outside of the nucleus also may be able to mediate the photoformation of reactive or toxic materials that could then diffuse into the nucleus and cause damage. The photodynamic treatment of fibroblasts with hematoporphyrin ultimately leads to a blurring of the nucleus, the disappearance of the nucleoli, and a multiple infolding of the nuclear membrane.⁵⁷ Nuclei in similarly treated lymphoma cells also degenerate; some become pyknotic and have clumped chromatin and others exhibit chromatin that looks like a homogeneous, granular

mass.⁷⁹ Chromatid breaks in metaphase chromosomes occur in WI-38 human lung cells illuminated in the presence of neutral red, acridine, and acridine orange dyes. These breaks may be mediated by the photodynamic release of lysosomal enzymes, because dyes like neutral red do not appear to penetrate into the nucleus.⁸⁷ DNA extracted from human skin fibroblasts that were treated with proflavine and then illuminated shows a significant decrease in average molecular weight as measured by an alkaline sucrose gradient technique. The breaks are repaired completely within 2 hr after illumination.⁸⁸ Essentially similar results are observed with skin fibroblasts from patients with xeroderma pigmentosum. Human tumor cells sensitized with hematoporphyrin and Chinese hamster cells sensitized with hematoporphyrin derivative also show alkali-labile lesions and single-strand breaks in their DNA when illuminated^{89,90}; repair is rapid in both cases. In the human tumor cells, the frequency of breaks is not increased in D₂O, but cell survival decreases significantly. This information, together with the observed rapid repair of photodynamically damaged DNA, suggests that DNA single-strand breaks play only a minor role in photodynamic cell inactivation. The rapidity of DNA repair observed in these experiments is interesting because many enzymes, including DNA-dependent RNA polymerases I and II, in mammalian cells are rapidly inactivated by photodynamic treatment.⁹¹ The illumination of human cells sensitized with hematoporphyrin also results in sister chromatid exchanges.⁹²

An immunofluorescence technique involving antibody specific for unpaired cytosine residues in DNA was used in studies that determined that guanines in the DNA of human cells are destroyed by photodynamic treatment with methylene blue.⁹³ Immunologic techniques were also used to show the specific destruction of DNA in different bands of human leukocyte chromosomes.⁹⁴

2.4.8. Immunologic Effects

Illumination of A positive red blood cells in the presence of protoporphyrin progressively decreases the reactivity of the cells with anti-A serum up to the point where hemolysis occurs.⁹⁵ Sarcoma 180 cells sensitized with thionine and 6C3HED tumor cells sensitized with acridine orange dye were illuminated until viability was lost; injection of these cells into host animals did not immunize them to subsequent challenge with untreated cells.⁹⁶ In contrast, immunization of rats with fibroblastic sarcoma cells that had been illuminated in the presence of acridine orange dye until their transplantability was lost did render the host animals immune to the subsequent implantation of viable tumor cells.⁹⁷

2.4.9. Anaerobic Photosensitized Reactions

Psoralens, as discussed earlier, form covalent photoadducts with DNA; this process does not require oxygen. Some psoralens also form covalent photoad-

ducts with certain proteins such as serum albumin in solution. The mechanism is different from that involved with DNA in that oxygen is required; furthermore, psoralens irradiated before the addition of protein also form adducts.⁹⁸ When irradiated under aerobic conditions, some psoralens produce singlet oxygen and can induce the photooxidation of amino acids and proteins.^{99,100} Most studies of psoralen-induced photoeffects on mammalian cells have been carried out under aerobic conditions. Thus, although it may be reasonably assumed that the photoresponses involving nuclear DNA result from anaerobic reactions, aerobic photoreactions involving proteins and other molecules cannot be excluded automatically.

Photosensitization with psoralens elicits various responses in mammalian cells,^{5,16-18,101} including the production of cross-links in nuclear DNA and RNA, cell killing (loss of colony-forming ability), blockage of cell division, inhibition of DNA and RNA synthesis, inhibition of virus growth in cells, alteration of nuclear fine structure, induction of sister chromatid exchanges, induction of mutations, and production of cells with altered morphology (cell transformation). Psoralens form photoadducts with the nucleic acids of cell nuclei.¹⁰¹ Bifunctional (cross-linking) psoralens, such as 8-methoxysoralen, photoinhibit mammalian cell colony formation much more efficiently than monofunctional psoralens, such as angelicin, because the cells repair cross-links much less efficiently than they do monoadducts. The latter are probably repaired by an excision repair system.^{102,103} With 4, 5', 8-trimethylpsoralen as sensitizer, UVA radiation specifically blocks the division of murine melanoma and human skin fibroblasts in the G2 phase.¹⁰⁴

In L1210 leukemia cells sensitized with psoralen, phototreatment strongly and equally inhibits DNA and RNA synthesis, presumably because psoralen-DNA photoadducts form that interfere with DNA template activity. With higher radiation doses, protein synthesis in these cells is also inhibited, perhaps by a photobinding of the sensitizer to ribosomes.¹⁰⁵ The photoformation of angelicin monoadducts with the DNA in monkey kidney CV-1 cells induces transcription termination lesions in the DNA that decrease the overall rate of RNA synthesis and also give rise to shorter-than-normal RNA chains. Repair of these lesions occurs, apparently by some type of excision repair process.¹⁰⁶ The phototreatment of human lymphocytes sensitized with 8-methoxysoralen inhibits DNA synthesis and also blocks the subsequent stimulation of DNA synthesis by phytohemagglutin P.¹⁰⁷ Phototreatment of mammalian cells sensitized with psoralens inhibits virus production in the cells; bifunctional psoralens are more effective than monofunctional derivatives.¹⁰² Treatment with 8-methoxysoralen plus UVA radiation causes alterations in many kinds of cells. Human epidermal cells treated with 8-methoxysoralen and UVA radiation exhibit nuclear abnormalities¹⁰⁸; human glia cells exhibit changes in the fine structure of the external cell membrane¹⁰⁹; human lymphoid cells show an increase in sister chromatid exchanges¹¹⁰; Chinese hamster V-79 and ovary cells show significant

increases in the frequency of mutations.^{111,112} Some cell lines show an altered morphology (transformation); hamster embryo cells transformed by this treatment give rise to tumors when implanted into newborn hamsters and cause spindle cell sarcomas in the cheek pouches of hamsters.¹¹³ The chemotactic response of human leukocytes is also significantly inhibited.¹¹⁴ Irradiation of sonicated DNA in the presence of 8-methoxysoralen produces psoralen-DNA adducts that can induce specific antibodies; such antibodies may be useful in detecting psoralen-DNA photoadducts in cells of patients receiving psoralen plus UVA radiation (PUVA) therapy.¹¹⁵

A few types of molecules, other than psoralens, sensitize cells to photodamage by anaerobic mechanisms. For example, chlorpromazine induces the anaerobic photolysis of red blood cells.^{6,116} Hemolysis also occurs if the chlorpromazine is first irradiated and then added to the cells. Lysis appears to be mediated by a stable, detergent-like anaerobic photoproduct of the sensitizer. Preirradiated chlorpromazine is also toxic to macrophages. In contrast, chlorpromazine and other phototoxic phenothiazines sensitize liposome membranes to photodamage by reactions that require oxygen.¹¹⁷ Protriptyline, a phototoxic antidepressant drug, also sensitizes red blood cells to photolysis under anaerobic conditions by a mechanism similar to that of chlorpromazine.¹¹⁶

2.4.10. DNA Damage, Mutation, Transformation, and Carcinogenesis Induced by Photosensitization

Aerobic sensitized photoreactions produce reactive oxygen species including hydrogen peroxide (a chemical mutagen)¹¹⁸ and singlet oxygen (a base substitution mutagen).¹¹⁹ Alterations in DNA, chromosome breaks, and sister chromatid exchanges are produced in photosensitized reactions with both aerobic sensitizers and psoralens. Thus, photosensitization probably induces mutations in mammalian cells. One case of mutation has been reported with an aerobic sensitizer. Illumination of V-79 Chinese hamster embryo cells in the presence of rose bengal covalently bound to hydrophilic polystyrene beads (Sensitox II—this sensitizer remains outside the cells) induces ouabain-resistant mutants.¹²⁰ The photodynamic mutation rate is increased by the tumor-promoting agent 12-O-tetradecanoyl-phorbol-13-acetate and by D₂O and is decreased by β-carotene and by the singlet oxygen trap 1,3-diphenylisobenzofuran. The D₂O and inhibitor data suggest that mutation production is mediated by reactions involving singlet oxygen. UVA irradiation of V-79 Chinese hamster cells in the presence of chlorpromazine in air significantly increases the frequency of mutations from 8-azaguanine sensitivity to 8-azaguanine resistance. Similar mutations with respect to 6-mercaptopurine sensitivity also occur. Under the same conditions, there is a significant increase in chromosome aberrations after irradiation. Whether oxygen is necessary for these effects has not been deter-

mined.¹²¹ As pointed out above, mutations are also induced in cells by illumination with fluorescent lamps in reactions presumed to be sensitized by endogenous chromophores; psoralens also induce photomutation.

Mammalian cells in culture are transformed when irradiated in the presence of psoralens and when illuminated with fluorescent lamps in the absence of added sensitizers, as already described. There are apparently no reports of cell transformation in culture sensitized by added aerobic sensitizers. However, the transformation of mouse cells by UV-radiation-inactivated type II herpes simplex virus is enhanced when the cells are subjected to a low-level photodynamic treatment with proflavine as the sensitizer.¹²²

Tumors can be induced experimentally in animals by photosensitized reactions. This was first reported in 1937 by Büngeler,¹²³ who demonstrated that the subcutaneous injection of the aerobic photosensitizers hematoporphyrin or eosin into albino mice followed by exposure to sunlight resulted in the induction of tumors. Neither the photosensitizers nor the sunlight exposure alone gave rise to tumors. More recently it has been shown that visible-light (tungsten-bulb) illumination of mice treated with neutral red or proflavine results in tumor formation.¹²⁴ The mechanisms by which tumors are produced photochemically with these types of sensitizers are not known, although theories of tumor initiation involving singlet oxygen have been advanced.¹²⁵ In some cases, as discussed above, cells transformed by psoralen plus UVA radiation treatment form tumors when implanted into animals. Tumors can also be induced in psoralen-treated animals by UVA irradiation^{124,126}; again, nothing is known of the mechanisms involved.

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Chapter 3

Experimental Techniques in Photoimmunology

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3.1. Introduction

Exposing an experimental sample to nonionizing radiation is similar in some ways to adding a chemical reagent to the sample, except that photons instead of molecules are being added. Just as interpretation of chemical experiments requires that one know the nature and amount of the chemical reactant, interpretation of photobiologic experiments requires that one know the characteristics and amount of radiation given. The distribution of energy at different wavelengths, irradiance, and total exposure dose are important factors. The sequence and times of exposure in relation to other events are also important. In this chapter, we will give a practical introduction to optical radiation sources and their measurement as they are used in photoimmunologic research. Excellent, more general discussions of this topic have been published elsewhere.¹⁻³ Detailed information on sources and radiometry^{4,5} and specific manufacturer's data^{6,7} are also available.

3.2. Terminology

This section describes the appropriate terminology used when discussing radiant electromagnetic energy in the optical portion of the spectrum. There have arisen, over more than a century, many terms and measurement schemes to quantify optical radiation. Confusion can be avoided by considering the simple and precise physical meaning of various radiometric terms and by thinking of radiant energy and its measurement in the same basic physical context.

Energy, in this case radiant energy, is defined and measured as an ability to do work in the classical physical sense and is measured in joules. Ideally, if one

joule of optical radiation is absorbed by an object, its temperature rises exactly as much as if one joule of thermal energy were added to the object. The term, *radiant energy*, is used to designate the ability of electromagnetic radiation to pass through a vacuum. As discussed in Chapter 1, more energy is delivered by a shorter wavelength photon than by a longer photon ($E = hc/\lambda$). Radiation can be measured either by the numbers of photons delivered or by the energy delivered by the photons. In either case, no measurement would be complete without specifying the distribution of the photons or energy over the various wavelengths present. In the vast majority of applications, energy units (joules) rather than numbers of photons are used. In some measurement schemes, energy is measured by absorbing the radiation on a black-surfaced object and then by measuring the precise temperature rise of the object. Thermopiles and pyroelectric detectors work this way. In other schemes, photons are the units being counted; for example, in photomultiplier tubes, the current generated by the tube is proportional to the number of photons arriving at the cathode and not to the energy that they collectively deliver.

Power is defined as the rate at which energy is transferred from one site or kind to another and is measured in watts. Conveniently, one watt equals one joule per second: The radiant power of a light source in watts is the number of joules emitted per second (J/sec). Stated another way, the radiant energy (E) in joules that a source can deliver in a certain time is equal to the radiant power (P) in watts times the time (t) in seconds: $E = P \times t$.

To specify wavelength distribution, the word *spectral* can be added to any power or energy term, and units are expressed per nanometer. Thus, the spectral power of a source is measured in watts per nanometer (W/nm). A plot of spectral power versus wavelength is therefore a basic and quantitative description of the distribution of power at different wavelengths and is usually available upon request from manufacturers of sources. The total radiant power of a source is the sum of the spectral power for each wavelength present, i.e., the integral or area under the spectral power curve. Similarly, the integral of the spectral power curve between any two wavelengths gives the radiant power in just that portion of the spectrum. This, very simply, is the advantage of spectroradiometry: One can document the wavelength distribution of power or energy and, from that distribution, know the radiation delivered within any precise waveband of interest.

Generally, surfaces are exposed to radiant energy at a given distance from the source and with a given geometrical arrangement relative to the source. Several terms describe the energy in power which reaches a surface from a source. *Radiant exposure dose* means the radiant energy delivered per square unit of surface area. The term *fluence* is sometimes used synonymously with radiant exposure dose in older literature. *Irradiance* describes the radiant power delivered per square unit of surface area. The terms *intensity*, *dose-rate*, or *flux*

are often used synonymously with irradiance, although intensity has a more precise meaning in terms of power emitted into a unit solid angle by a source. The radiant exposure dose (D) in J/cm^2 equals the irradiance (I) in W/m^2 times the time of exposure (t) in seconds: $D = I \times t$. Knowing the spectral irradiance curve (in $\text{W/m}^2 \times \text{nm}$) in a particular experimental setup and the exposure time in seconds, one can calculate the radiant exposure dose delivered over any wavelength region. The spectral irradiance of a source and particular experimental setup is usually measured in order to calculate the exposure times needed to give the desired radiant exposure doses. Scanning spectroradiometers can be used to accurately measure spectral irradiance, and other detectors that respond to broad or narrow spectral bands are often used to measure only that portion of the spectral irradiance that falls within wavelengths of particular interest.

3.3. Sources of Nonionizing Radiation

The sun is a powerful source of nonionizing radiation, providing 1300 W/m^2 of 259- to 3000-nm radiation at noontime on earth (see Fig. 1.3 in Chapter 1). However, the sun is usually not a practical source for experimentation because its irradiance and spectral distribution vary drastically with time and location. Artificial sources are available with differing spectral power distribution, total power, and geometrical configuration. The execution and interpretation of experimental results requires the choice of an appropriate light source. The source should be known or designed to meet the specific goals of the experiment. A large range of useful types of artificial Ultraviolet (UV) and visible radiation sources are available, including common light bulbs that emit mostly visible radiation, fluorescent and mercury-discharge sunlamps that emit UVB radiation, and high-power arc lamp systems and lasers that are suitable for special purposes. Some of the more commonly used sources are discussed here.

Electromagnetic radiation from most light sources includes a range of wavelengths, either in a continuous spectrum or in discrete emission lines. Most of the artificial sources useful in experimental photobiology are classified as gas discharge lamps. This category includes germicidal lamps, fluorescent tubes, and xenon arc lamps. The radiation emitted by these sources results directly or indirectly from the excitation of gas molecules contained within a glass or quartz envelope by electrical current. Collision between the electrons and gas molecules causes the gas to ionize. Subsequent recombination of the electrons with the nuclei releases electromagnetic energy. The wavelengths emitted are characteristic of electronic transition energies for the gas in the lamp and are often modified by temperature and pressure. At low gas pressures, narrow line spectra at predominantly shorter wavelengths are produced; that is, energy is emitted at only a few specific wavelengths.

3.3.1. Germicidal Lamps

Germicidal lamps are a common example of a low-pressure gas discharge lamp. They contain mercury at low pressure and emit about 85% of their energy at 253.7 nm, usually referred to as 254-nm radiation. The rest of the energy is emitted as lines at several other wavelengths in the UV and visible spectrum. The envelopes of germicidal lamps are made of quartz or special UV-radiation-transmitting glasses. The chief advantages of low-pressure mercury germicidal lamps are (1) their simplicity and (2) the high irradiance at an emission line, 254 nm, which is absorbed by many aromatic chromophores that cause marked photobiologic responses. The vast majority of *in vitro* photobiologic research has been conducted with 254-nm radiation simply for these reasons. The disadvantages are that (1) the output of 254-nm radiation varies with the temperature of the lamp, (2) the lamps must be spectrally filtered when only 254-nm radiation is desired, and (3) the 254-nm radiation wavelength does not occur naturally at the earth's surface. Safety precautions in using germicidal lamps are imperative: Eyes exposed for a few seconds to the unfiltered bulb, which typically produces 10 W/m^2 irradiance at 254 nm, develop photokeratitis, because exposure to only 50 J/m^2 of 254-nm radiation can cause this response.

3.3.2. Fluorescent Lamps

Common fluorescent lamps are glass-enveloped low-pressure mercury discharge lamps in which a phosphor coating is applied to the inside of the envelope. Phosphors are mixtures of inorganic compounds that absorb the 254-nm radiation and reemit the energy at longer wavelengths. The wavelength of the reemitted energy is determined by the specific phosphor coating the inside of the tube. The envelope is usually made of soda-lime glass that almost entirely absorbs any 254-nm radiation not absorbed by the phosphor, but transmits the longer wavelengths emitted by the phosphor.

A wide variety of fluorescent lamps with emission bands varying in width from tens to hundreds of nanometers are available. The spectral power distribution varies among bulbs of the same type made by the same or different manufacturers. The main advantages of fluorescent lamps are their (1) simplicity, (2) variety, (3) interchangeability, (4) low cost, and (5) (usually) their safety. The main disadvantages are that (1) the output of the lamps diminishes over time as the phosphor degrades, (2) the maximum irradiance achievable is typically much less than 100 W/m^2 , and (3) like the germicidal lamps, the output varies with the temperature of the lamp. The irradiance at the surface of fluorescent bulbs is roughly constant along their length and their emission is diffusely directed. Careful attention should be paid to providing a uniform irradiance over the samples. Samples placed distant from or under the ends of fluorescent lamps are exposed to lower irradiance than samples centered under the lamps.

Two frequently used UV-radiation-emitting fluorescent lamps are the Westinghouse FS sunlamp or UVB fluorescent lamp, with maximum spectral irradiance near 315 nm and useful output from 275 to 350 nm, and the black light or UVA fluorescent lamp, typically with maximum output at 344 nm. The total UV irradiance near the lamps is approximately 5 to 10 W/m² for the sunlamp and 30 to 100 W/m² for the UVA fluorescent lamp. Very little energy is emitted by the UVA fluorescent lamp at wavelengths less than 300 nm; however, for many photobiologic effects, including lethality and mutagenesis, and skin erythema and pigmentation, these shorter wavelengths may be 1000 times more effective than 360-nm radiation. Consequently, a filter, such as Mylar, must be used for critical work to remove shorter wavelengths.

3.3.3. Xenon Arc Lamps

The spectrum produced by high-pressure xenon arc lamps is broad and, with the exception of the near-infrared region, strongly resembles extraterrestrial sunlight. This occurs because the gas plasma of the arc is approximately the same temperature as the surface of the sun. Solar simulation may therefore be accomplished using a xenon arc lamp in combination with glass absorption filters that mimic the atmospheric absorption of UV wavelengths by ozone and also appropriately decrease the infrared proportion. Several such solar simulators are commercially available. Xenon arc lamps and most other high-power compact lamps pose a retinal burn hazard and can cause permanent blindness similar to "eclipse blindness" from viewing the sun.

High-pressure xenon-mercury and mercury compact arc lamps emit a continuous spectrum with sharp spikes at the spectral emission lines of mercury. Xenon, mercury-xenon, and other compact arc lamps have the distinct advantage of being nearly point sources, which allow their radiation to be focused into beams or very intense spots. High-pressure xenon or mercury-xenon arc lamps are often used in conjunction with a monochromator, which selects a narrow wavelength band of irradiation from the broad emission spectrum. Such narrow bands are necessary for determining photobiologic action spectra. For this reason, and because of xenon's broad emission spectrum, the expense and maintenance of compact arc xenon or mercury-xenon sources used with a monochromator or filters are often justified.

3.3.4. Other Radiation Sources

Other radiation sources such as incandescent bulbs and lasers are less commonly used, but can be useful in specific experimental situations. Lasers are especially useful for their monochromaticity, power, and capability of producing very brief intense pulses and high collimated beams.

3.4. Spectral Filtering of Sources

A radiation source rarely emits precisely the desired spectral energy distribution, and, therefore, optical filters are often necessary. Any material that absorbs, reflects, or scatters optical radiation can act as an optical filter, and unintentional filtering must always be considered. For example, the petri dish covers or the medium lying over cells in tissue culture, and even the spectral reflectance of the tabletop beneath the cells will affect the dose and spectral distribution of optical radiation reaching the cells.

When using sources of UVA or visible radiation, one must often ensure that even small amounts of the highly biologically active wavelengths less than 320 nm are not present at the site of irradiation. For this purpose, glass filters and several plastic polymers which absorb short wavelengths but transmit longer wavelengths are useful. Optical glass filters available for this purpose (Schott, Corning, and Hoya are major suppliers) should be used when possible. Detailed transmission spectra are available for the various filter glasses made. For blocking shorter wavelengths, however, the series of sharp-cut optical glasses offered by Schott are particularly valuable. Optical glass filters are, in general, inexpensive and suitable whenever a low-to-moderate power, small-area source optical beam is filtered. For large filters, sheets of Mylar or other plastics and even ordinary window glass can be used. Most glasses and plastics are damaged photochemically by UV radiation, and their spectral transmission therefore changes with use. This damage must be monitored carefully by repeated measurement of spectral transmission, and the filters should be replaced as necessary. The optical characteristics of window glass and plastics are often determined by minor impurities; the composition and thickness of the materials can vary from one batch to another and from one brand to another.

The filtering of the intense beams of radiation produced by xenon or xenon-mercury compact arc lamp systems often present a special problem because optical absorption filters become hot enough to crack or melt. In general, filters always should be uniformly irradiated to avoid focal heating and thermally induced stress in the filter material. With most arc lamp systems over 1000 watts, liquid-cooled filter assemblies or the use of inorganic or organic liquid filters, which can dissipate large amounts of heat without damage, may be necessary.² An aqueous solution of copper sulfate makes an excellent filter that allows UVA and blue visible radiation to pass through, but blocks shorter UV wavelengths and infrared radiation. Nickelous and cobaltous sulfate together in aqueous solution allow excellent transmission of 230- to 320-nm radiation, but block most of the UVA, visible, and infrared spectrum. Another useful high-power filtering device is an all-dielectric coated dichroic mirror that reflects a bell-shaped band of wavelengths while transmitting most other wavelengths. Used as a selective reflecting mirror, most of the unwanted wavelengths can be

allowed to pass through the mirror. Dichroic mirrors are expensive, but can be ordered for maximum reflectance at any desired wavelength. A common and useful scheme is to grossly prefilter the beam by using liquid filters or dichroic mirrors that eliminate most of the power in unwanted spectral bands. This pre-filtering protects the final filtering element, which is generally an optical absorption glass filter, interference filter, or monochromator chosen to pass the precise waveband desired.

Very narrow wavebands are necessary for determining action spectra and are also useful for maximizing one particular photobiologic effect relative to some other competing or interfering photobiologic response. There are several means of obtaining monochromatic or nearly monochromatic radiation, the most common of which is to use a monochromator, generally with a xenon or xenon-mercury source, to selectively pass the desired band. Many excellent monochromator systems, handling various powers, are commercially available. The major advantage of a monochromator is that it can be tuned across the spectrum. Interference filters that consist of multilayered dielectric materials coated onto standard optical glasses are also available. The dielectric layers reflect all but a narrow band of wavelengths within the passband of the optical glass filter substrate; thus, this compact but expensive filter transmits only a narrow band of wavelengths around any chosen center wavelength. However, the filter has limited power-handling capabilities, is expensive, and, unlike a monochromator, cannot be tuned as easily across the spectrum.

The small amounts of stray radiation at wavelengths outside the waveband of primary interest are often important to know, but difficult to measure. Calculation of low stray radiation values can be achieved by careful measurement of the irradiance of the unfiltered source and of the spectral transmission of the filter used. Stray radiation is usually expressed as a ratio with respect to the irradiance of the wavelengths of interest.

3.5. Radiometry

The basic scheme in radiometry is to employ a stable device that converts photon absorption into a readily measured signal for the purposes of radiation measurement. Spectroradiometry measurements are needed with broadband sources to determine the wavelength distribution of the energy. Photometry refers to the measurement of visible radiation by detectors with the same spectral response as the human eye. Photometry is intended primarily for lighting purposes and is rarely useful for characterizing spectral radiation for nonvisual photobiologic studies. In radiometry, devices are used that absorb photons and produce readily measurable signals. Recent technological developments have produced stable instruments with a range of sensitivity, wavelength response,

and fields of view that allow reasonably well-calibrated, defined, repeatable measurements. Radiometers generally consist of a detector with filters and other “input optics,” and an electronic readout device. The broad categories of radiometric detectors that are currently available commercially are thermopiles or pyroelectric devices, photodiodes, and photomultipliers.

3.5.1. Types of Radiometric Instruments

3.5.1.1. Thermopiles

Thermopiles measure irradiance by absorbing all photons reaching the sensor (detector) and converting the energy to heat. The heat is then converted by means of thermocouple junctions into a voltage that is proportional to the energy of the radiation. Since thermopiles are equally sensitive to all wavelengths of light, a single thermopile detector can, in theory, be used to measure the irradiance of all light sources. Care must be taken when measuring UV radiation to adequately equip the thermopile with filters to exclude visible and infrared radiation, if necessary. The irradiance measurements obtained with a thermopile are meaningful only if the precise transmission spectra of such filters used are known. Without filters, thermopiles measure the total irradiance present, provided that the thermopile detector has a full field of view of the source. Temperature variations within laboratories sometimes affect thermopile measurements; hence, vacuum or sealed thermopiles tend to be more stable. Many types of thermopiles are available, ranging from delicate and extremely sensitive units to hermetically sealed, highly stable small units. Thermopiles are stable devices, can be calibrated to within a few percentage points of accuracy using standard sources developed by the U.S. Bureau of Standards, and are often used to calibrate other detectors or sources.

3.5.1.2. Photodiodes

Vacuum photodiodes are used as detectors in many commercially available UV radiometers. Exposure of a vacuum photodiode’s cathode to electromagnetic radiation generates electrons by the photoelectric effect, which when driven to the anode produces an electrical current. The current is directly proportional to the number of incident photons. In contrast to thermopiles, the wavelength sensitivity is limited at longer wavelengths by the energy required to eject electrons from the coating on the cathode. When properly calibrated, vacuum photodiodes are easy to use, relatively rugged, reliable, and highly sensitive.

Photomultipliers are simply vacuum photodiodes in which the electrons are amplified by a series of collisions with other electron-emitting stages. Photo-

multipliers are used whenever sensitivity is paramount, such as in analytical instruments, low-level sources, and spectroradiometers.

Solid state silicon photodiodes are now available that are inexpensive, accurate, and sensitive over the 250- to 1000-nm region. These simple detectors are rugged and readily incorporated into small, accurate radiometer systems. Most solid state detectors, however, lack the exquisite sensitivity inherent in photomultipliers.

3.5.2. Factors of Measurement

Regardless of the specific light source and radiometer, several practical factors must be considered before an accurate measurement of radiant energy can be obtained. The emission spectrum of the light source and the spectral response range of the detector must overlap in a quantitatively known way. For instance, to measure the intensity of fluorescent sunlamp tubes, which emit radiation mainly in the 290- to 350-nm range, a detector must be used which is sensitive to these wavelengths. A detector that is sensitive only to the 340- to 370-nm range will give an essentially meaningless measurement. More precisely, any detector and source combination will yield a measurement of irradiance equal to the spectral integral of the product of the source's spectral irradiance times the detector's spectral sensitivity. For precise work with broadband sources, spectroradiometer measurements of spectral irradiance, or very carefully chosen broadband detectors with a spectral response tailored to the measurement desired, are usually necessary. A spectroradiometer is a radiometer that uses a calibrated monochromator for selectively scanning across the wavelengths of interest. The irradiance in wavebands is calculated by integrating the corresponding area under the spectral irradiance curve. Broadband detectors are much simpler devices that consist of a detector and filter combination responding to relatively narrow wavebands (i.e., UVC, UVB, UVA, and visible). Spectroradiometers are more precise and versatile than broadband detectors; however, the latter are simpler and hence are used more often.

Accurate measurements of irradiance or spectral irradiance require that the radiometer or spectroradiometer be accurately calibrated, stable, and responsive to the precise waveband of interest, and that it include in its calibrated field of view all radiation striking the detector from all angles. This latter factor can lead to very large quantitative errors when a narrow-field-of-view radiometer is used to measure wide-area sources, such as fluorescent lamps, skylights, or banks of lamps. Because the contribution to the total irradiance produced by a ray striking a flat surface varies as the cosine of its angle of incidence, so-called "cosine-corrected" radiometer-input optics are necessary for accuracy with wide-area sources.

Once the proper radiometer, spectroradiometer, or thermopile is chosen, several practical aspects must be borne in mind: (1) The measurement must be

obtained at the precise position of the sample or subject. Without focusing optics, the intensity of a lamp generally decreases as the distance from it increases. For point sources, such as compact arc lamps, the familiar inverse-square law holds. The precise decrease in irradiance with distance from a particular source depends on the dimensions of the source. For banks of fluorescent lamps, for example, the decrease is approximately zero very near to the lamps, then nearly linear for some distance, and finally falls off as the square of the distance from the lamps. (2) The intensity of most arc or discharge lamps fluctuates shortly after they are ignited. Thus, measurements should be made only after the lamp output has stabilized, typically after a few minutes. (3) The uniformity of irradiance over the exposure field area should be determined. In some cases, rotating the samples or the source during exposure can help to attain more equal exposure doses in all samples. (4) The measurement should be made at the site of light absorption. For example, if animals in cages are being irradiated, the detector must be placed in the cage so that it is shielded in the same way as the animals' skin. If cells in dishes or flasks are being irradiated, the measurement must be made through the plastic or glass container. (5) The distribution of radiation within the sample must be considered. This is especially true if action spectra or quantum-yield determinations are to be done with any accuracy. Several simple means exist for correcting for optical scattering by cells irradiated in stirred suspension.¹ Even a monolayer of cells typically absorbs half of the 254-nm radiation striking the layer, such that wavelength corrections for absorption of cells in multiple layers or in tissue become necessary.

Despite the potential technical problems and errors involved in assuring that the exact desired spectral irradiance and exposure dose have been delivered in an experiment, the laboratory practice of reliable radiometry has, as its reward, the ability to reproduce the results and often to understand one's results in the greater context of photobiological science.

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Tissue Optics and Photoimmunology

R. Rox Anderson

4.1. Introduction

Whenever an organism, organ, or cell is exposed to UV or other optical radiation, the response, or lack of response, depends to some extent on the optical properties of the exposed tissues. Delineating the exact nature of this dependence is both necessary and instructive in experimental work. For example, cells in tissue culture are more sensitive to loss of viability by exposure to 254-nm radiation when they are adherent and flattened against their substrate than when they are rounded. In the latter case, a greater pathlength of cytoplasm intervenes between the plasma membrane and nucleus, resulting in more effective shielding of the DNA chromophore by cytoplasmic absorption and scattering. One can, in fact, use this to measure the optical absorbance of living cytoplasm.¹ Thus, one of the first concerns in the study of tissue optics is to ascertain the fraction of incident radiation at specified wavelengths that reaches important chromophores and initiates responses, or perhaps for the immunologist, that reaches the tissues and cells mediating immune responses.

In the case of human skin, an extremely wide range of depths within tissue are achieved by different optical wavelengths, with shorter wavelengths, in general, penetrating more superficially. If the action spectrum for a photoimmunologic response is fairly broad, it is possible to choose wavelengths that either maximize or minimize doses delivered to different cells of special interest. For example, Langerhans cells in the epidermis are always exposed to larger doses of optical radiation than are underlying, circulating, or wandering lymphocytes, monocytes, or plasma cells. However, choices of wavelength, skin type, and exposure conditions can be coordinated to affect a specific kind of cell. For instance, a long duration exposure of pigmented skin to a short wavelength should, in theory, maximize the relative dose to Langerhans cells because (1) most melanin pigment in the skin lies beneath Langerhans cells, (2) shorter wave-

lengths penetrate superficially compared with longer wavelengths, and (3) circulating cells are exposed only for the duration of their transit through skin.

Precise knowledge of tissue optics is also necessary for rational comparison of photoimmunologic phenomena elicited or discovered *in vitro* with those obtained *in vivo* and *in situ*. Ultraviolet radiation has been shown both *in vitro* and *in vivo* to modulate membrane markers, to alter antigen processing or presentation, and to be lethal to peripheral blood lymphocytes. These effects and the validity of extrapolations from *in vitro* studies to *in vivo* responses can only be correlated well if one can measure or calculate the doses received by specific cell types *in vivo* with reasonable accuracy.

The skin is an especially dynamic organ, well-suited for its primarily protective role. Part of this activity includes inducible hyperplasia and hyperpigmentation, both of which profoundly affect its optical properties. Quantitative modeling of skin is further complicated by a marvelous heterogeneity of structure and composition. A useful experimental model of skin tissue optics should be neither too complex nor too simple and must include or allow for (1) dynamic variability, spanning the time scale from essentially instantaneous changes in blood volume and flow, to reactive hyperpigmentation of hyperplasia, to senile atrophic changes; (2) multiple layers with entirely different pigments, thickness, structures, and functions; and (3) a clean separation of the two basic optical processes governing radiation transfer, namely scattering and absorption.

It is not the purpose of this chapter to dwell upon mathematical models of optical radiation transfer in skin, although several very interesting approaches to this problem have been taken.²⁻⁶ Rather, the chapter presents a useful overview of basic concepts and measurements necessary to the study of tissue optics and presents a relatively simple model with data typical for human skin. As is almost always the case with physical measurements in biology, precise work requires one to perform optical measurements and calculations for the specific tissues, animals, or subjects being investigated, rather than to rely upon values reported in the literature.

4.2. The Stratum Corneum and Epidermis

4.2.1. Structural Considerations

The keratinocytes in the compact, adherent, cellular squamous epithelium called the epidermis are dedicated to an orderly death, as they migrate outwards, differentiate, lose their nuclei, flatter, and die to produce the outermost, poorly permeable, tough, and protective stratum corneum. At the base of the epidermis and attached to a basement membrane are the stem cells of the basal cell layer, only a fraction of which are actively dividing at any one time. Dendritic melanocytes make up only a small percentage of the cells along the basal layer but

produce and inject into keratinocytes all of the melanin pigment granules (melanosomes, about 1 μm in size), the number and aggregation of which determine racial skin colors and profoundly affect optical absorption within the epidermis. In both blacks and whites, most of the melanin present is in the resting basal cells, although a significant amount migrates outward with keratinocytes. There are normally 6 to 10 cell layers in the epidermis and in the stratum corneum over most of the body, but the epidermis is typically 60 to 100 μm thick, whereas the stratum corneum is only 8 to 15 μm thick and is of greater density. From an optical point of view, the greater concentration of proteins within the stratum corneum and the greater concentration of melanin within the basal layer make the epidermal compartment of the skin heterogeneous. The epidermis is entirely cellular and lacks large refractile fibers, such as are present in connective tissue; this lack of fibers may contribute to the decreased amount of optical scattering seen in epidermis as compared with the underlying dermis.

The stratum corneum has a refractive index (n_D) near 1.55,⁷ and about the same value in the near-UV radiation wavelengths.⁸ The corneocytes of the stratum corneum are tightly cohesive and sebaceous, and other lipids normally coat the skin, in essence providing a single optical interface with the air. Thus, about 4.6% of perpendicularly incident optical radiation (by Fresnel's equation) is reflected by the surface of the stratum corneum. Actually, this regular reflectance component is more typically between 5 and 6% because of irregularities in the surface topology of the stratum corneum; it may also vary with hydration or the composition of surface film.⁶ In psoriasis, there are numerous superficial air spaces near the skin surface and flakes of stratum corneum are visible as white scales because the corneocytes are poorly adherent, abnormal in structure, and are rapidly formed. The multiple air-skin interfaces cause markedly increased reflection of all optical wavelengths. Application of oils that more nearly match the refractive index of the stratum corneum causes a striking and immediate broad-spectrum reduction in skin reflectance.⁹ Presumably, the same mechanism is responsible, in part, for the increased efficacy of UVB radiation therapy of psoriasis when oils are applied before each exposure.

4.2.2. Scattering

Within any of the layers of skin, 95% of the incident radiation is not returned by regular reflectance, but is absorbed or scattered (Fig. 4.1). These two processes taken together essentially determine the penetration of radiation into skin. Scattering results from inhomogeneities in a medium's refractive index, corresponding to physical inhomogeneities, i.e., structures. The spatial distribution and intensity of scattered light depends upon the size and shape of the inhomogeneities relative to the wavelength and upon the difference in refractive

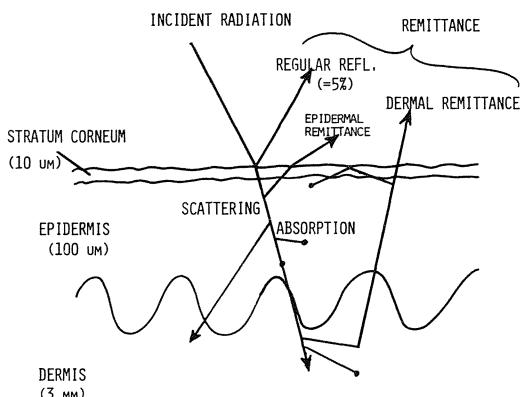


Figure 4.1. Schematic diagram of optical pathways in skin.

index between the medium and the inhomogeneities. For molecules or small particles with dimensions less than roughly one-tenth the wavelength, scattering is generally weak, nearly isotropic (equally distributed spatially), polarized, and varies inversely with the fourth power of wavelength (Rayleigh scattering). For particles with dimensions on the same order as the wavelength, scattering is much stronger, more forward-directed, and although varying inversely with wavelength, is not such a strong inverse function. When the particle size greatly exceeds the wavelength (so-called Mie scattering), scattering is again diminished and becomes highly forward-directed. Within the skin, all of these general types of scattering occur. However, scattering by structures with dimensions on the order of optical wavelengths or somewhat larger is more common than Rayleigh scattering. In particular, scattering by collagen fibers appears to be very important in determining the penetration of optical radiation within the dermis, and scattering in the epidermis is of less importance, in general, than absorption in determining the penetration of epidermal UV and visible radiation. Scattering is most marked in the UV portion of the spectrum.

4.2.3. Absorption

Absorption of UV and visible radiation in tissue corresponds to electronic excitations of aromatic or conjugated unsaturated chromophores. A large and unknown number of chromophores is present in skin, but several major chromophores account for most of the optical absorption within each skin layer. In the stratum corneum and epidermis, these are melanin, proteins containing aromatic amino acids, urocanic acid, and nucleic acids. Within the stratum corneum, carotenoids are also important chromophores. Each of these chromophores has unique absorption spectra, different biologic importance, and different distribution within the tissue. Absorption spectra of major UV chromophores of the epidermis and stratum corneum are shown in Fig. 4.2, and typical transmission

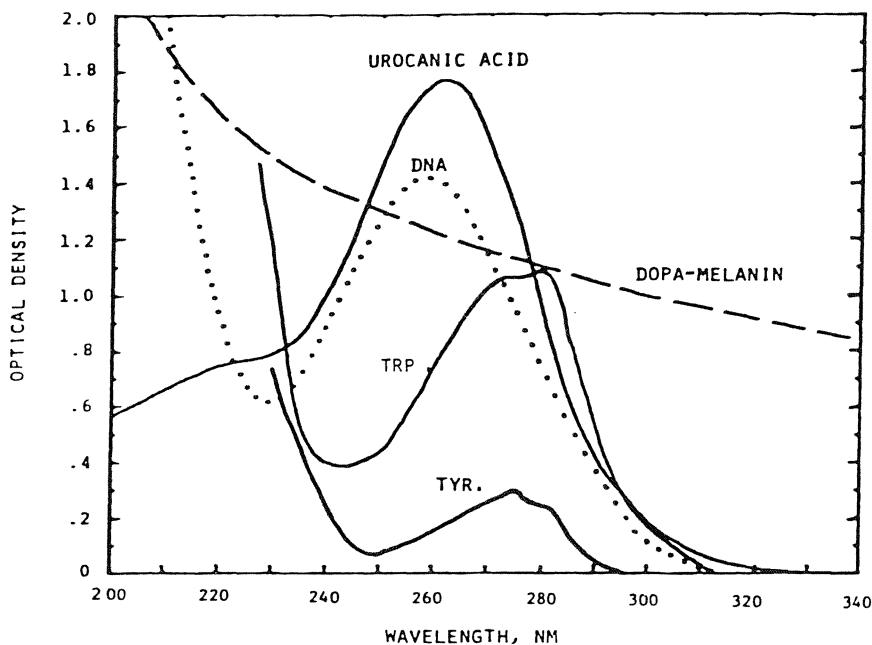


Figure 4.2. Ultraviolet absorption spectra of major epidermal chromophores. DOPA-melanin, 1.5 mg% in H₂O; urocanic acid, 10⁻⁴ M in H₂O; calf thymus DNA, 10 mg% in H₂O (pH 4.5); tryptophan (TRP), 2 × 10⁻⁴ M (pH 7); tyrosine (TYR.), 2 × 10⁻⁴ M (pH 7).

spectra, taken with an integrating sphere spectrophotometer and corrected for tissue fluorescence, are shown in Fig. 4.3.⁶ It can be seen that epidermal or corneal transmittance spectra are compositely determined by absorption by these (and certainly other) substances.

Transmission of UV and visible radiation through the stratum corneum or epidermis of fair-skinned Caucasians qualitatively resembles that through protein

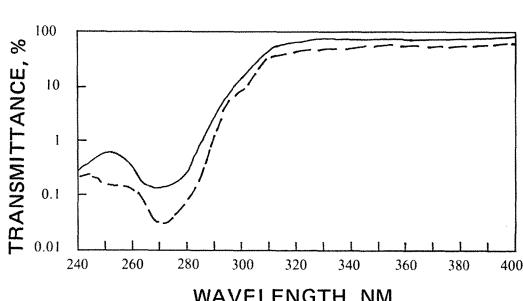


Figure 4.3. Transmittance spectrum of fair-skinned surgically obtained heat-separated Caucasian epidermis (---), and of stratum corneum (—) separated by streptococcal scalded skin syndrome epidermolytic toxin, from an adjacent site on the anterior thigh. The spectra are total forward transmittance measurements free of any fluorescence error.

containing the aromatic amino acids tryptophan and tyrosine. A minimum in transmittance occurs near 275 nm (Fig. 4.3). Nucleic acids, with an absorption maximum near 260 nm, and urocanic acid, with an absorption maximum at 277 nm at pH 7.4, also contribute to the broad 275-nm absorption band of epidermis and stratum corneum. The content and distribution of melanin usually plays a major but highly variable role in determining the transmission of optical radiation through the stratum corneum and epidermis depending upon the genetically determined capacity of an individual for constitutive and facultative pigmentation. The high absorbance of epidermis and stratum corneum for wavelengths around 200 nm is largely due to peptide bonds.

Many standard spectrophotometer systems allow the options of an integrating sphere or other scattered radiation measurement accessories. Since the advent of these devices, several groups have measured and published total transmission spectra of human epidermis^{10,11}; of particular interest are those given by Everett et al.¹⁰ Measurements of tissue transmittance are often complicated, however, by a broad fluorescence excitation band centered near 280 nm that is associated with an emission band between 330 and 360 nm and is consistent with tryptophan or tyrosine fluorescence.⁶ This emission band can be sufficient to cause errors in the epidermal transmission spectra that are determined by spectrophotometers equipped with broadband photomultipliers (essentially all standard spectrophotometer systems). This autofluorescence error can be overcome by using a “solar-blind” detector, which is insensitive to wavelengths longer than 320 nm. A second problem arises if the tissue is suspended in air or placed against a quartz slide, typically at the entrance port of an integrating sphere. There is some total internal reflection at the interfaces of the sample, resulting in the loss of some off-axis rays and, hence, a measurement error. This can be largely overcome by using normal saline solution as the optical medium on the dermal side of epidermal samples. The saline also maintains the samples in an environment more similar to that *in vivo*.

Variations in the concentrations, distributions, or amounts of epidermal chromophores and in the thickness of the epidermis largely determine individual and anatomic variations in epidermal spectral transmission. One might expect the protein- and nucleic-acid-bound chromophores to be of rather constant concentration and distribution in normal skin because these chromophores are inherent to all cellular tissue. Both melanin and urocanic acid, however, have variable concentrations and distributions, and unlike protein or nucleic acid, optical absorption may be their major function in human skin.

4.2.3.1. Melanin

There are conflicting reports on the possible photoinduction of urocanic acid synthesis in the epidermis^{12,13} but melanin is certainly photoinducible. In

the visible portion of the spectrum, melanin is essentially the only pigment that affects the transmittance of normal human epidermis, giving rise to the wide range of discernable skin colors from "black" to "white." The 300-nm transmittance of full-thickness, suction-separated epidermis including the basal cell layer varies by 2 to 3 orders of magnitude from very fair Caucasian skin types to darkly pigmented Negroid skin. Melanin is not a "neutral density" filter of the skin; its absorption increases steadily toward shorter wavelengths over the broad spectrum of 250 to 1200 nm. At near-infrared wavelengths longer than about 1200 nm, the optics of skin are essentially unaffected by melanin pigment. This is readily appreciated in diffuse reflectance spectra of Caucasian and Negroid skin *in vivo* (Fig. 4.4), in which the diffuse reflectances are essentially identical beyond 1100 nm. There are many unsolved and interesting biologic questions

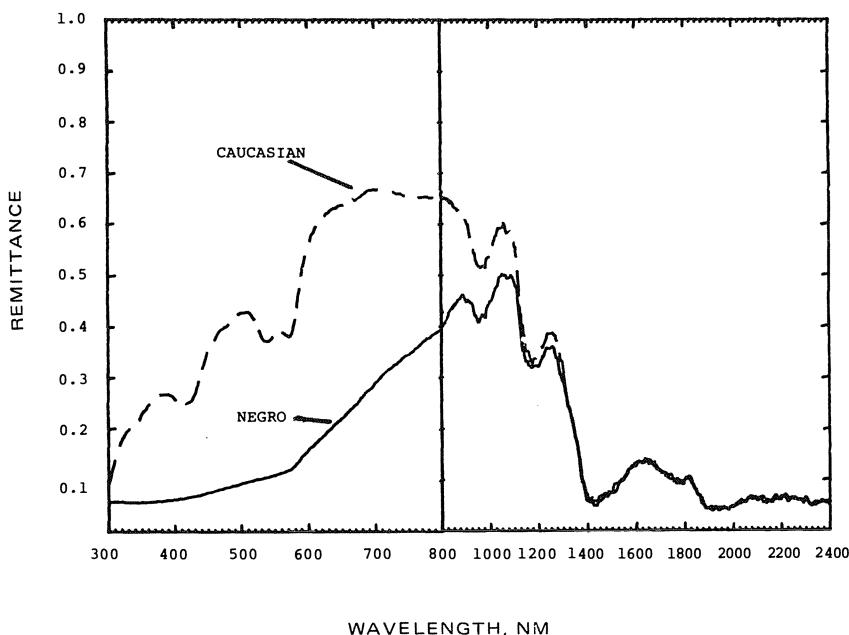


Figure 4.4. Spectral diffuse reflectance of Negroid and fair Caucasian skin (flexor surface of forearm in each case). The lack of significant absorption by melanin for wavelengths longer than approximately 1100 nm and the increased absorption at shorter wavelengths are apparent. Note also that diffuse reflectance is never less than 5% in either case because of regular reflectance at the skin surface.

regarding melanogenesis, and even the basic role of the melanin polymer is open to question. In Caucasian skin, the action spectrum for delayed melanogenesis is nearly identical with that for delayed erythema over most of the UV spectrum,¹⁴ but it is possible to induce hyperpigmentation without erythema by administering multiple daily small doses of UVA radiation.^{15,16} This poses questions of whether the cascades leading to hyperpigmentation and inflammatory phases of response to UV radiation share identical primary chromophores, target sites or cells, and, partially, mechanisms, or whether melanogenesis is secondary to the preceding inflammatory phase. Ultrastructural evidence of melanocytic mobilization of melanosomes within minutes after exposure and well before clinical evidence of inflammation¹⁷ argues strongly for specific and direct induction of increased melanocyte activity by UV radiation. In a broader sense, the well-known photoprotection and coloration offered by epidermal melanin may not be the primary role of melanin. Other functions are suggested by its presence in both mushrooms and the human brain. Trapping reactive free radicals, mediating other redox reactions, and acting as a semiconductor¹⁸ have been proposed as roles for melanin in different tissues, or it may simply be a fortuitous byproduct of other reactions.

Photoprotection by melanin is well known to those who have compared their ability to withstand solar exposure before and after tanning of the skin. However, a certain fraction of photoprotection results from the hyperplasia that is also induced by exposure to UV radiation, primarily UVC and UVB wavelengths. For wavelengths less than 310 nm that are absorbed well by nonmelanin epidermal chromophores, hyperplasia alone offers some photoprotection, but protection against longer wavelengths is related to total melanin content and distribution. With chronic exposure, a negative feedback "loop" is established in which both the melanogenic and hyperplastic responses diminish further actinic damage and a new equilibrium state of skin is eventually established. Although details of these basic photoprotective responses are poorly understood, certain observations are germane to a discussion of tissue optics. Melanin is a chemically stable protein-polymer complex, the chromophoric backbone of which survives attack by proteases, acids, and bases. Caucasian melanosomes typically contain a greater number of melanin granules, but less total melanin than Negroid or Mongolian melanosomes. Caucasian melanosomes also appear to undergo greater degradation within keratinocytes. The optical effects associated with dispersed "melanin dust" in Caucasians versus intact melanosomes have not been quantitated, but it is likely that, unless the chromophoric backbone is degraded, dispersal of melanin pigment in Caucasian stratum corneum affords somewhat greater protection than would the same quantity of melanin sequestered in intact melanosomes. An interracial study of epidermal transmittance by Kaidbey et al.¹⁹ suggests that the large (ten- to thirtyfold) racial differences in sensitivity to UV radiation^{20,21} correlate poorly with the small (approximately threefold) racial

differences noted in stratum corneum transmission. However, the minimal erythema dose of black and white subjects has never been directly compared with accurate measurements of the same subjects' stratum corneum or epidermal transmittance at various levels in the tissue.

4.2.3.2. Urocanic Acid

Urocanic acid is thought to play some role as an "endogenous sunscreen" of the epidermis and stratum corneum,^{22,23} but is certainly of less photoprotective importance than melanin or proteins. The epidermis lacks significant urocanase activity, and, hence, urocanic acid is accumulated. Extraction of water-soluble, diffusible, UV radiation-absorbing compounds from skin into topically applied water accounts for the up to 50% increased sensitivity of skin to UVB radiation after the skin has been hydrated for prolonged periods. Although most of the material extracted is lipid or protein, a small fraction (about 0.2% by weight) of the material is urocanic acid.²⁴ However, because of its high extinction coefficient ($18,800 \text{ liter M}^{-1} \text{ cm}^{-1}$ at 277 nm, pH 7.4), urocanic acid accounts for approximately 75% of the UV radiation absorbance of the extracted materials. Absorption by urocanic acid extends well into the spectral region associated with natural sunburning reactions. Extensive exposure to sunlight is often associated with sweating, which deposits urocanic acid on the skin surface. It is possible that sweating may serve to some extent as a thermally induced photoprotective mechanism.

4.3. The Dermis

4.3.1. Structural Considerations

The dermis is mesodermal in origin and typically 2 to 4 mm thick, i.e., 20 to 60 times the thickness of the overlying epidermis. It is composed mainly of highly refractile collagen fibers with interspersed fibroblasts, vessels, nerves, and lymphatics. The normally small numbers of mast cells, macrophages, lymphocytes, and granulocytes present can be markedly increased by immunologic or inflammatory stimuli. The optics of the dermis are very different from those of the epidermis, reflecting the differences in structure and pigments of these layers. There is a comparative paucity of aromatic amino acids in dermal protein, 70% of which is type I collagen. No melanin is normally present, urocanic acid is rapidly metabolized, and the major dermal pigments are entirely intravascular (hemoglobin, oxyhemoglobin) or are in equilibrium with the circulation (bilirubin) and subcutaneous fat (carotenoids). Because of its circulation and its position as a site for the expression of inflammatory reactions, the optics of the

dermis can be highly dynamic. The vessels of the uppermost or papillary dermis are layered into superficial and deep venous plexi, with a superficial arterial plexus intervening.

4.3.2. Scattering

The importance of optical scattering in dermis is readily appreciated. In 1956, Hardy et al.²⁵ found that the Beer-Lambert law was invalid for dermis by studying the spectral transmittance of sequential thick sections of Caucasian and Negroid skin, suggesting either the heterogeneity of structure or the presence of significant scattering. In 1970, Findlay²⁶ reported that thin sections of dermis or dura mater of newborns showed a gradually increasing diffuse reflectance toward the shorter (blue) wavelengths, but gradually increasing transmittance toward the longer (red) wavelengths. This, plus the fact that little absorption was noted, is best explained by strong optical scattering, which varies inversely with wavelengths over the visible spectrum. Subsequently, Anderson et al.^{6,27} analyzed measurements of diffuse spectral transmission and reflection of human dermal sections *in vitro* for the purpose of determining absorption and scattering coefficients. The radiation transfer model used was a modification of the basic Kubelka-Munk analysis,²⁹⁻³⁰ which has, in general, been a popular analysis for skin optics^{2,3,6,31,32} and other turbid media. Results of this analysis showed that the dermal scattering coefficient, S , of this model varies inversely with wavelength and exceeds the value of the corresponding absorption coefficient, in the absence of blood (Fig. 4.5). Thus, it is apparent that the optical scattering in the dermis plays a major role in limiting the penetration of UV and visible radiation into skin and also helps account for the observation that longer wavelengths penetrate connective tissue much better than do shorter wavelengths.

4.3.3. Absorption and Optical Penetration

In vivo, the blood-borne pigments, hemoglobin, oxyhemoglobin, β -carotene, and bilirubin are the major absorbers of visible radiation in the dermis. Absorption spectra for these pigments are shown in Fig. 4.6, along with that for "DOPA-melanin" (autopolymerized 3,4-dihydroxyphenylalanine). The penetration of visible and near-UV radiation and, to some extent, shorter UV wavelengths within the dermis depends in part on the state of vasodilation present. This is especially true in the 400 to 600 nm range (Fig. 4.6). The oxygen saturation of blood also affects dermal optics, and the blue hue of hypoxic skin is an expression of both reactive hyperemia and the relative deletion of red wavelengths by reduced hemoglobin versus oxyhemoglobin. However, for the more biologically and immunologically active UV wavelengths, the melanin content and thickness

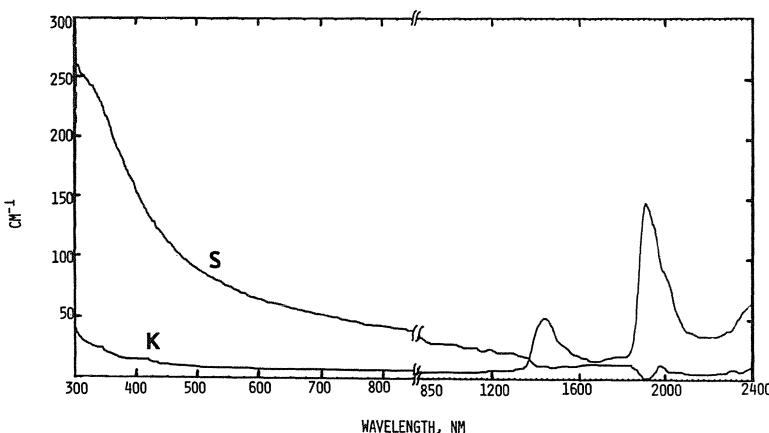


Figure 4.5. Diffuse scattering (S) and absorption (K) coefficients for human dermis in vitro, calculated from measurements of diffuse spectral reflectance and transmittance of thin dermal sections under conditions appropriate to application of the Kubelka-Munk theory of radiation transfer.²⁷

of the epidermis and the scattering properties of the papillary dermis are the major factors limiting penetration into the tissue.

Despite several attempts to directly measure spectral radiation levels within living skin, calculations based on in vitro data and models of radiation transfer currently offer the best quantitative generalizations regarding the penetration of optical radiation into skin. Table 4.1 shows the approximate depth in very fair skinned, living Caucasians (i.e., essentially no melanin present) at which radiation of various wavelengths is attenuated to various fractions of the incident energy density. The estimates shown in the table are derived by summing the values for fluxes in the Kubelka-Munk model³⁻⁶ as calculated assuming that the scattering coefficients are known from in vitro studies^{6,27} and that diffuse in vivo reflectance can be used to estimate dermal absorption coefficients. It is difficult to firmly establish the accuracy of the values given in Table 4.1 because of the lack of reliable invasive in vivo measurements, the variability among individuals and sites, and the multiple simplifying assumptions necessary to model the transfer of radiation in vivo. However, the values given are largely consistent with previous data or other estimates.^{2,3,5,25,32,33-35}

Measurements of spectral reflectance are one of the few noninvasive optical measurements obtainable in human skin. Despite many uncertainties, it is possible to estimate the transmittance of epidermis *in vivo*² from diffuse reflectance spectra. Further possibilities for analysis of reflectance spectra include bilirubin content³⁶ and hemoglobin oxygen saturation.³²

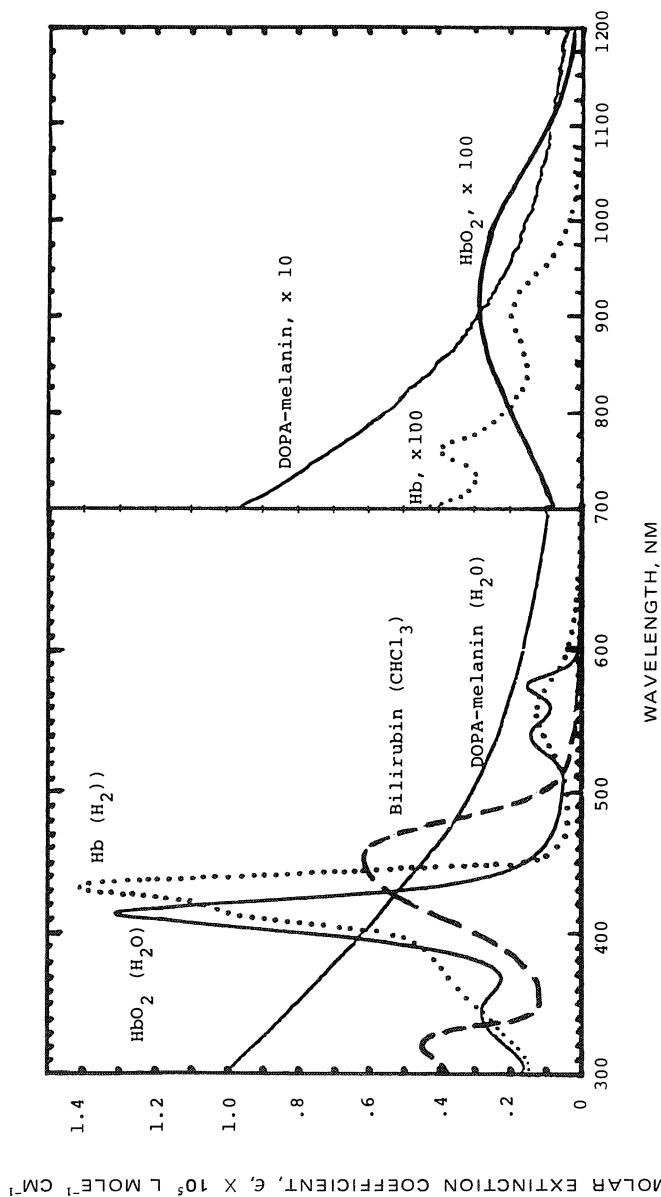


Figure 4.6. Absorption spectra of major pigments of human skin: HbO_2 (—), Hb (····), Hb (—·—), HbO_2 (—·—), and DOPA-melanin (—). Parentheses indicate solvent. The values plotted for Hb and HbO_2 spectra are per mole of heme iron and are therefore one fourth of the values for molar extinction expressed for hemoglobin tetramers. The spectrum shown for DOPA-melanin is the absorbance on a scale of 0 to 1.5 of 1.5 mg/dl aqueous solution. Not shown is β -carotene, which has a broad absorption band in the 400- to 500-nm region, with maxima at 466 and 497 nm in CHCl_3 . Note that the scale changes in the near infrared.

Table 4.1
Estimated Optical Penetration into Very Fair Human Skin

Wavelength	Approximate depth of incident energy density decrease (μm)			
	37%	10%	1%	0.1%
250	2	4.5	9	14
280	1.5	3.5	7	11
300	6	14	28	42
350	60	140	260	380
400	90	210	240	450
450	150	350	700	1100
500	230	530	1100	1600
600	550	1300	2500	3800
700	750	1700	3400	5200 ^a
800	1200	2700	5400 ^a	8100 ^a

^aSubcutaneous.

4.4. Summary and Conclusion

The optics of skin are dynamic, variable, and complex but can be analyzed quantitatively if certain simplifying assumptions are made. In the epidermis, melanogenesis and hyperplasia account for protection against wavelengths less than 310 nm, and the presence of aromatic amino acids, urocanic acid, and nucleic acids is evidenced by a broad epidermal absorption maximum (minimum in transmittance) near 275 nm. At 275 nm, Caucasian stratum corneum of normal thickness typically has a transmittance between 0.1 and 1%, but this value may be as high as 5% in some cases. Negroid stratum corneum may transmit $\frac{1}{3}$ to $\frac{1}{10}$ this amount at 275 nm. At all optical wavelengths longer than 320 nm, melanin is the major photoprotective chromophore, and nonpigmented epidermis offers little barrier to these wavelengths. Melanin's differential concentration within resting basal cells at the base of the epidermis causes marked optical absorption in this layer for pigmented skin. As a result, keratinocytes and Langerhans cells overlying the basal cell layer are exposed to much greater relative doses of radiation than are circulating cells or dermal cells. Although increased melanin pigmentation reduces doses for all cells, the disparity between suprabasal versus subbasal doses becomes greater with increasing pigmentation. The exact amount, packaging, and distribution of melanin are genetically influenced and form an environmentally determined equilibrium.

The percentage of incident radiation reaching the dermis is highly dependent upon the wavelength, the thickness of the epidermis (for $\lambda < 310$ nm),

and the melanin content (for $\lambda > 200$ nm). In contrast, optical penetration into the dermis is determined mainly by the scattering of collagen fibers and by absorption by blood (for $\lambda = 400$ -600 nm). As is true for the epidermis, longer wavelengths, in general, pass relatively deeper into the tissue and an optical "window" into the tissue exists beyond 600 nm. Table 4.1 gives estimated depths for different energy densities within the tissue as a function of wavelength for fair Caucasian skin. The approximate doses of radiation experienced by fixed cells depends on the wavelengths present and on states of pigmentation, hyperplasia, and vaso-dilatation; for circulating cells, the dose received is the integral of the radiation density at the level of superficial vessels over the time during which the circulating cell passes through the tissue. Thus, if a given surface exposure dose is delivered in a brief period by a powerful source, the maximum dose per cell to the minimum number of circulating cells will be delivered. If the same total surface exposure dose is given over a longer period, a lower dose per cell will be received by a large number of circulating cells. For fixed tissue cells, the doses received will be the same in each case.

If the optical characteristics of tissue are considered together with the mechanisms by which optical radiation affects immune competent cells, it may be possible to design *in vivo* experiments to precisely study immune responses and ultimately to design new therapies.

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Chapter 5

Acute Effects of Ultraviolet Radiation upon the Skin

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5.1. Introduction

Light and UV radiation hitting the skin cause various biologic effects, many of which can be harmful. Some of the important barrier functions of the skin serve (1) to minimize damage by injurious agents such as radiation, (2) to repair the damage that occurs, and (3) to enable the skin to undergo adaptive changes that protect the organism damage from future exposures.

Apart from the synthesis of vitamin D, which is the only useful interaction between the skin and UV radiation so far identified, the acute responses of the skin to UV radiation may be broadly divided into inflammatory, reparative, and protective responses. The initial phase of damage results in inflammation, with the classic signs of redness, swelling, and pain. This is followed by repair at molecular and cellular levels, and then by adaptive changes, thickening of the epidermis and stratum corneum and increased melanization, which confer increased protection against subsequent irradiation. Chronic effects, such as premature aging of the skin, result from cumulative damage induced by years of exposure to the sun. Atrophic changes occur in the epidermis and connective tissue and result in wrinkling, telangiectasia, and increased skin fragility. The increased incidence of skin cancer in sun-exposed areas of fair-skinned humans is probably also partly the result of cumulative UV-radiation-induced damage to the nuclear material of epidermal cells caused by chronic exposure to the sun.

Although considerable information is available about the acute changes occurring at molecular, pharmacologic, microscopic, and gross clinical levels after UV irradiation, the interrelationships are still poorly understood. For example, although many photochemical events directly resulting from UV irradiation have been identified in the skin, the initial photochemistry as well

as most of the “dark” chemical reactions and pharmacologic events in the cascade leading to erythema are still unknown.

In this chapter, the short-term effects of UV irradiation upon the skin will be reviewed.

5.2. Erythema

5.2.1. Introduction

Erythema is the most conspicuous cutaneous response to nonionizing radiation in man. It is an indication of increased blood volume in the superficial and deep vascular plexi in the dermis and, in Caucasian skin, becomes visible when the volume of blood increases by a mean of 38% over normal levels.¹ Detectability of erythema varies according to the degree of constitutive or facultative pigmentation present.

Erythema is detected easily in fair-skinned individuals and can be monitored easily over a period of time after the skin is injured by UV radiation. As a result, erythema has been widely used as an indicator of inflammation in the study of cutaneous responses to UV radiation. Erythema can be induced by a single dose of UV radiation of appropriate wavelength. The minimum erythema dose (MED) is usually defined as the least energy required to produce clearly marginated erythema in the irradiated area of skin. Although somewhat subject to observer variation, this end point has, by and large, proved valuable and fairly reproducible and is sufficiently accurate to act as a guide for therapeutic UV radiation dosages given to the whole body. An alternative end point that is sometimes employed is the energy dose causing minimum perceptible erythema, rather than causing a well-defined area with sharp margins. In either case, the figure is expressed as energy delivered per unit area, for example mJ/cm^2 and J/m^2 . It does not define the irradiance or the duration of administration.

Most photobiologic responses occur in proportion to the total energy, or number of photons, delivered and not in proportion to the rate of administration; doubling the irradiance and halving the time of irradiation therefore elicits the same magnitude of response. This reciprocal relationship between intensity and time, sometimes simply called reciprocity, holds for the delayed erythema component of inflammation over a wide range, up to 7 orders of magnitude.^{2,3} Reciprocity may break down under some circumstances; for example, when extremely low irradiances are given, repair mechanisms may keep pace with photon-induced damage. At very high irradiances, thermal effects may predominate over the usual photochemistry because of the finite rate at which heat can be dissipated. Even if the initial photochemistry shows strict reciprocity, the observed biologic response may not do so because of rate-limiting steps or saturation of pathways in the intervening cascade of events.

5.2.2. Time Course

In experimental animals, such as the rat or guinea pig, an immediate phase of erythema, that fades within 15 min, follows exposure to UVB radiation; this phase is associated with increased vascular permeability⁴ either related to direct vascular damage⁵ or mediated through mast cells.⁶ In man, an immediate phase is not normally observed. Erythema usually begins several hours after UVB irradiation and reaches a maximum between 12 and 24 hr later. When intense erythema is induced (for example, by administration of 4 to 8 times the minimum erythema dose of UVB radiation), the response begins earlier, takes longer to peak, and is more persistent. The color fades over a period of several days, blending with other later changes, such as delayed tanning or peeling of the skin, that may follow sufficient exposure. In humans, some individuals show more persistent erythema than others; this feature has been claimed as a marker for predisposition to actinically induced skin cancer^{7,8} and does not appear to correlate with the ease with which erythema is induced (MED value). However, in another study, erythema was found to be more persistent in individuals with fair skin (a known risk factor for cancer), after 5 times the MED of solar stimulator radiation.⁹ Individuals with the heritable disorder that affects DNA repair, xeroderma pigmentosum, also show persistent erythema and have a high incidence of actinic skin cancers. The time course of erythema may also vary with wavelength: Shorter wavelengths (UVC) tend to induce a more rapid response that peaks sooner and is less intense at 24 than at 8 hr. UVA-radiation-induced erythema is maximal between 12 and 24 hr.

Under certain conditions in man, the erythema response may have two phases. An early or immediate phase begins during radiation and is maximal during radiation or shortly afterwards; this early phase partially or completely fades and is followed by delayed erythema. This phenomenon is seen mainly after the administration of experimental long-wavelength (UVA) radiation. Since UVA radiation is much less erythemogenic than shorter wavelengths, high doses are required to induce erythema (about a thousandfold greater than that required for UVB radiation). With radiation being administered over a short time, the resulting thermal load on the skin is considerable and may be partly or wholly responsible for the immediate phase of erythema, which does not occur if UVA radiation is given more slowly. Immediate erythema induced by UVA radiation may be related to irradiance and not only to total exposure dose.

As well as differences in time course, there are also differences in dose-response relationships in different spectral regions. A dose of 10 to 20 times the MED of UVC radiation causes only a slight increase in erythema and scaling, and normal skin can easily tolerate $100 \times$ MED. In contrast, a relatively modest increase (6 to 8 times the MED) in the dose of UVB or UVA radiation causes intense dusky erythema, sometimes with edema and blistering, that takes several weeks to heal. Five times the MED of noonday sun (which is polychromatic

UVB radiation, UVA radiation, and longer wavelengths) may also cause severe erythema and blistering.

5.2.3. Factors Influencing the Development of Erythema

5.2.3.1. Wavelength

For the initial photochemistry to take place in the cascade of events leading to the delayed erythema, photons must both penetrate to and be absorbed by the appropriate chromophore. Both of these processes are wavelength dependent. Many biomolecules are known to be altered by UV radiation, but a specific target that will trigger erythema when damaged has not been identified. A single target, or chromophore, may not exist, and cells may be damaged in many ways. The pyrimidine bases of nucleic acids and the amino acids of proteins are believed to play an important role. *In vitro*, these and other molecules maximally absorb radiation in the UVC range, which is not present in terrestrial sunlight, but they also significantly absorb in the biologically more important UVB range.

The relative effectiveness of different wavelengths of radiation in the induction of erythema is expressed as the erythema action spectrum, obtained by plotting the reciprocal of the lowest exposure dose required to obtain a certain grade of erythema response versus wavelength. For minimum erythema, the most erythemogenic wavelengths are in the 250 to 290 nm range of the UV spectrum. Due to a rapid decrease in effectiveness as the wavelength increases to 320 nm, a thousand times as much energy is required at 320 nm than at 290 nm to cause erythema.¹⁰ Figure 5.1 shows the action spectra for erythema and for melanogenesis in fair-skinned humans. The difference in the 8-hr and 24-hr erythema action spectra at shorter wavelengths (UVC) reflects the more rapid time course of erythema induced by this spectral region. The shape of the erythema action spectrum curve resembles the curve obtained if the transmission of radiation by the stratum corneum at different wavelengths is multiplied by the absorption spectrum for nucleic acids. This is to be expected if nucleic acids are in fact a significant chromophore in erythemogenesis. The shape of the action spectrum curve in part depends upon the end point adopted, since the dose-response relationships⁵ differ with wavelength. If, for example, the energy dose for intense erythema rather than minimal erythema was measured, the shorter (UVC) wavelengths would appear to be relatively less effective. Differences in erythema time course in relation to wavelength also help to account for differences between erythema action spectra measured using different intervals between exposure and observation of the skin.

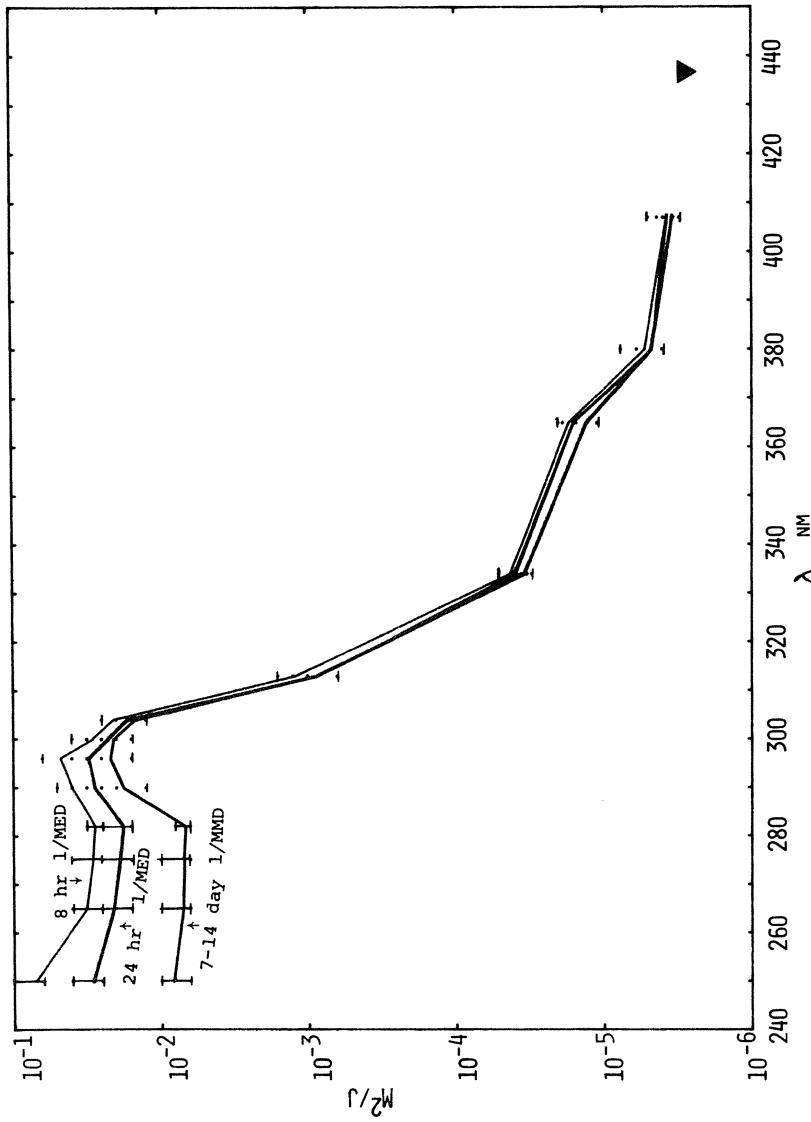


Figure 5.1. Action spectra for erythema (read at 8 hr and 24 hr after exposure) and melanogenesis (read at 7 days after exposure) in fair-skinned humans. (MMD) Minimal melanogenic dose.

5.2.3.2. Pigmentation

Skin color is another important factor in determining erythemogenesis. Resistance to the induction of erythema by UV radiation is conferred by both racial pigmentation and by facultative tanning. Fair-skinned people require 3 to 5 times less UVB radiation to induce erythema than people with moderately pigmented skin and up to 30 times less than people with darkly pigmented skin. Facultative tanning is less photoprotective; when people were tested after 3.5 months of exposure to summer sun, investigators found that the MED of tanned skin were an average of 2.3 times greater than that of untanned (buttock) skin.¹¹ Four weeks of intensive UVB radiation treatment for psoriasis can cause an eight fold rise in MED.¹¹ The photoprotective effect of facultative tanning is partly the result of increased cutaneous melanin and partly the result of increased stratum corneum thickness; the relative lack of protection afforded by a UVA-radiation-induced tan¹² may be partially attributable to the relatively small concomitant increase in stratum corneum thickness characteristic of UVA-radiation-induced tanning.

5.2.3.3. Other Factors

Differences in MED noted in different parts of the body and seasonal variations in MED are probably related to differences in pigmentation and skin thickness. Age may also play a role; the MED for young children and elderly people is lower. The effects of atmospheric conditions, such as heat, humidity, and wind, upon the induction of erythema in humans have not been clearly defined.

5.2.3.4. Waveband Interactions

Additive effects of UV radiation of adjacent wavelengths are a fundamental assumption in most studies employing any sources other than strictly monochromatic ones, such as lasers. By increasing the bandwidth of a filter or monochromator system, the energy delivered is increased, presumably also increasing biologic responses. However, when the effects of simultaneous or consecutive radiation from different parts of the UV spectrum are examined, more complex interactions may need to be considered.

While investigating possible protective effects of UVA-radiation-induced immediate pigment darkening against induction of erythema by subsequently administered UVB radiation, Willis et al.¹³ found that, in contrast to the expected protective effect, preirradiation of a site with UVA radiation actually increased the sensitivity to UVB radiation, as shown by a much increased erythema compared with sites not preirradiated. This interaction was considered greater than could be accounted for by an additive effect and was not order dependent, since it occurred to an equal degree if UVA irradiation followed

UVB. This phenomenon is termed photoaugmentation. Other studies of interactions between UVA and UVB have generated results more consistent with the concept of a directly additive effect¹⁴⁻¹⁶; when fractions of MED of UVA and of UVB radiation are administered to the same site, erythema will result if the sum of the fractions exceeds unity. This is described as photoaddition. This apparent additive effect is surprising considering the molecular and histologic differences in the effects of UVA and UVB radiation on skin.

Another type of interaction between different wavelengths has been termed photorecovery. van der Leun and Stoop¹⁷ showed that skin exposed to glass-filtered sunlight (UVA radiation and light) immediately after UVB or UVC irradiation showed a diminished erythema response (an increased erythema-dose threshold) compared with a site receiving no filtered sunlight. Similar findings were obtained by van Weelden.¹⁸ Filtered sunlight given first, before UVB or UVC irradiation, did not have this effect. Analysis of the interaction between carefully graded fractions of MED of UVA and UVB radiation administered to the same sites showed that slight photorecovery occurs in some individuals.¹⁶ The partial reversal by longer wavelengths of erythema induced by shorter wavelengths (UVB or UVC radiation) has not been consistently reproducible perhaps because of the small (20%) alteration in the dose threshold induced and because of the occurrence of simultaneous photoaddition.

The concept of photorecovery of erythema is interesting less for its practical significance than for the insight that it provides into the pathogenesis of erythema. The thymine-thymine dimer is produced extensively after irradiation of DNA with UV radiation, and plays a major role in the mutagenic and lethal effects of UV radiation in micro-organisms. This lesion may be reversed by photoreactivating enzyme in human tissue exposed to long-wave UV radiation or visible radiation (320 to 700 nm)¹⁹⁻²⁰ as well as by dark repair.²¹ If an analogous reversal of erythema by long-wave UV or visible radiation could also be demonstrated, this would support a role for DNA as a major chromophore and the thymine-thymine dimer as an important photoproduct in erythema-genesis.

Radiation may also interact additively with previously administered doses. For example, suberythemogenic doses of UVA and UVB radiation administered repeatedly at 24-hr intervals eventually have a cumulative effect resulting in erythema.^{22,23} After a single erythemogenic dose of UVA or UVB radiation, the erythema-threshold is reduced in the treatment area for at least 24 hr (L. Arbabi, unpublished observations).

Most studies of waveband interactions in skin have evaluated erythema. Attempts to demonstrate photoaddition or photoaugmentation of sunburn cell production,²⁴ photocarcinogenesis,¹⁰ or changes in epidermal DNA synthesis or polyamine biosynthesis²⁵ have been unsuccessful. This is probably a reflection of the small responses, if any, to long-wave UV radiation alone at the dose tested, making simple additive effects undetectable.

5.2.4. Solar Erythemogenesis

Although UVA radiation is much less erythemogenic than shorter wavelength UV radiation, the high UVA irradiance present in sunlight means that, in reality, long wavelengths contribute significantly to the sunburn reaction. UVA radiation may represent 15% of the erythemally effective radiation at noon.¹⁰ This proportion may be greater at other times of day, because, with increasing solar zenith angle, the atmospheric attenuation of UVB radiation is greater than that of UVA radiation, leading to a relative increase in the contribution of the longer wavelengths.

5.3. Pathogenesis of UV-Radiation-Induced Erythema

Several mediators believed to be important in the pathogenesis of UV-radiation-induced erythema have been identified. Studies have been performed in various animal species, including man; however, the results cannot always be carried over from one species to another. The initial photochemistry in the cascade leading to inflammation occurs in the epidermis with subsequent diffusion of mediators into the neighborhood of blood vessels. However, injury of dermal cells or structure or direct injury to blood vessels may also be important.⁵

Histamine has been identified as a potential mediator in delayed UV-radiation-induced erythema in humans as well as in the immediate erythema that is seen in the guinea pig and certain other species. A fourfold rise in histamine levels in human suction blister fluid has been demonstrated at the onset of UVB-radiation-induced erythema.²⁶ At the same time, partially degranulated mast cells were observed. Histamine levels returned to normal 24 hr after radiation. Previous studies employing dermal perfusion gave inconsistent results.²⁷ Since histamine causes vasodilation and increased permeability, it may be an important mediator in the earliest part of the response; however, since its effects are short lived and levels are normal after 24 hr, other mediators must be involved, at least at later times. Furthermore, antihistamines or histamine depletion do not detectably suppress the delayed erythema induced by UV radiation.²⁸ Increased levels of serotonin, a mediator also released from mast cells, have been shown in the urine of humans after UV irradiation.²⁹ Involvement of mast cells is also supported by the increased levels of prostaglandin D₂, predominantly a mast cell product, found in human skin after UVB radiation.³⁰

Ultraviolet-radiation-induced lysosomal disruption, with release of enzymes leading to cellular damage, may be an early step in the inflammatory process.³¹ The biphasic response seen in some experimental animals may be caused by endothelial cell lysosomal damage in the immediate phase, followed by the delayed effect of epidermal lysosomal disruption, and the diffusion of proteinases

and mediators down to the level of the blood vessels. However, cells severely damaged by UV radiation can contain intact lysosomes,³² and ultrastructural studies have shown early epidermal cell damage before visible lysosomal disruption is present. These findings, however, do not preclude a role for lysosomal disruption as an important event in the later phases of erythema.^{33,34}

A firm relationship between increased prostaglandin synthesis and UV-radiation-induced erythema has been demonstrated. Prostaglandins, in addition to directly causing vasodilation after intradermal injection,³⁵ may potentiate the effects of other mediators. Increased levels of prostaglandins are found in dermal perfusates from UV-irradiated skin²⁷ and in suction blister fluid obtained in the first 24 hr after UVB and UVC irradiation.³⁶⁻³⁸ The exact site of the increase in synthesis is not known in UV-radiation-induced inflammation, but most cellular constituents of skin, including epidermis, blood cells, and vascular endothelium, are known to be capable of prostaglandin synthesis. Indomethacin, a potent inhibitor of the cyclo-oxygenase pathway and, therefore, of prostaglandin biosynthesis, is effective in inhibiting the initial portion of UVB-radiation-induced delayed erythema.^{39,40} After 24 hr, however, prostaglandin levels return to normal, whereas erythema is usually still present and is unresponsive to topical inhibitors of prostaglandin synthesis. In the later phases of delayed erythema, other mediators are more important, but a role for prostaglandins synthesized by locally invading leukocytes has not been excluded. Erythema induced by UVA radiation is unresponsive to topical indomethacin,⁴¹ another important difference from the erythema induced by shorter wavelengths. This is also true for erythema induced by 8-methoxypsoralen followed by UVA radiation.

Mediators may also be formed in the plasma exudate that occurs during inflammation. Bradykinin, which may cause pain, erythema, increased vascular permeability, and leukocyte migration, has been demonstrated in the skin just before the onset of UV-radiation-induced erythema,⁴² but detection of kinins by other investigators has been inconsistent.²⁷

5.4. Histopathologic Changes Associated with UV-Radiation-Induced Erythema

Histologic changes can be seen in human skin as little as 30 min after irradiation, several hours before erythema develops.²⁶ In general, erythemogenic doses of UVB and UVC radiation cause both epidermal and dermal changes, whereas longer wavelengths (UVA) cause predominantly dermal changes.

Sunburn cells are a characteristic feature of UVB- and UVC-radiation-induced epidermal damage, although similar changes can also be seen in unrelated conditions. Sunburn cells result from damage to keratinocytes; they exhibit either swollen or shrunken cytoplasm that becomes hyaline and often

brightly eosinophilic; nuclear alterations also occur, leading to pyknosis and premature disappearance of the nucleus. In humans, sunburn cell formation may be observed within 30 min of erythema radiation,²⁶ peaking at 24 hr. Sunburn cells may also be visible following suberythemogenic doses of radiation. In animal studies, a somewhat longer latent period has been described⁴³; detectable numbers of sunburn cells were found 5 hr after irradiation in the mouse. After exposure to 300-nm radiation, a gradual increase in numbers with a peak at 24 hr occurred; exposure to 254-nm radiation induced a more rapid response that peaked at 8 hr and fell significantly by 18 hr.

Sunburn cells characteristically occur in irregularly distributed clusters and are formed mainly in the lower half of the epidermis; neighboring keratinocytes may be normal in appearance. After high doses, the cells may coalesce into a band of necrotic tissue that is eliminated upwards over a period of 7 days or longer. The reason(s) for the susceptibility of particular epidermal cells, and not others, to UV-radiation-induced damage and to the formation of sunburn cells is not understood; decreased DNA repair capacity,⁴⁴ increased melanin content,⁴⁵ lysosomal breakdown, or cell-cycle specific sensitivity⁴⁶ have been proposed.

The action spectrum for sunburn cell formation has been studied in the mouse.⁴³ Wavelengths of less than 300 nm were the most effective; no significant differences were observed over the range of 260 to 290 nm. After sensitization with topical 8-methoxysoralen, sunburn cells were induced by 325 to 425 nm radiation.

Other acute changes seen in the epidermis include intercellular edema, nuclear alterations, with nucleolar prominence in otherwise minimally altered cells, and occasional exocytosis of lymphocytes. Fewer recognizable Langerhans cells are found in human skin as soon as 1 hr after irradiation²⁶; even fewer are seen at 24 and 72 hr, and they are frequently vacuolated. Reduced Langerhans cell density is well-documented in mice⁴⁷ and man⁴⁸ after UV radiation⁴⁷: A normal population density is restored by 15 days.⁴⁹ Melanocytes also show vacuolization and swelling soon after irradiation of human skin, but tend to regain their normal morphology by 24 hr.

UVB irradiation also alters the dermis in various ways. The most striking acute changes occur in the blood vessels of the superficial vascular plexus. Endothelial cells enlarge within 30 min of irradiation, leading to partial occlusion of vessels that can be seen for several days. There is also striking perivenular edema in the early stages of the response in humans, perhaps corresponding to the early increase in vascular permeability shown in animal studies⁴ using trypan blue dye. Although the changes are most striking in the superficial dermis in humans, endothelial swelling also occurs in the deeper levels and even in the subcutaneous fat. A pervascular infiltrate is seen in man consisting predominantly of mononuclear cells around the more superficial vessels; this is most con-

spicuous after 2 to 3 days. Few neutrophils are seen; this contrasts with findings in the guinea pig, in which a striking invasion occurs, maximal between 4 and 16 hr, and that seems to be important in the pathogenesis of erythema in this species.⁵⁰ Animals pretreated with cyclophosphamide at an appropriate interval for the induction of leukopenia at the time of UVB irradiation required significantly greater energy for the induction of threshold erythema, which also lasted for a shorter period. This effect was not seen with UVC or UVA irradiation.

In humans, the number of mast cells decreases 1 to 4 hr after UVB irradiation²⁶; hypogranulated or degranulated cells are visible, sometimes with extracellular granules in the neighborhood. By 24 hr after irradiation, mast cells have regained their normal appearance and density.

The erythema induced by UVA radiation differs strikingly from that induced by UVB radiation in the balance which exists between the observed histologic changes and in that it requires much more energy for induction. For comparable degrees of erythema, dermal changes after UVA irradiation are more pronounced than after UVB irradiation⁵¹ with a denser mononuclear cell infiltrate extending to greater depths in the dermis. Vascular damage is also severe, with greater degrees of endothelial swelling and red cell extravasation. Not surprisingly, the changes are also more persistent. Ultrastructural studies⁵² showed wide gaps between endothelial cells, damaged pericytes, platelet aggregation in the lumina, and perivascular fibrin deposition.

Oral 8-methoxysoralen plus UVA radiation induces prominent changes in both epidermis and dermis that are more persistent than those seen after UV irradiation alone.⁵¹ Obvious changes were still present 7 days after exposure.

5.5. Pigmentation

Ultraviolet radiation also leads to an increase in epidermal melanin pigmentation, which is a protective response of the skin against further radiation. The pigmentation response occurs in two strikingly distinct phases, immediate and delayed, that have different action spectra. Although delayed tanning has been shown to be photoprotective, a protective function of immediate tanning has not been demonstrated.

5.5.1. Immediate Tanning

Immediate pigment darkening (immediate tanning) begins during irradiation and is maximal immediately afterwards, fading within minutes after small exposures, or lasting several days after larger exposures to radiation. The response is inducible by UVA and visible radiation⁵³ and has been observed in cadaver skin and in isolated melanosomes. A dose of 1 to 12 J/m² UVA radiation is sufficient

to induce immediate tanning in Caucasians who tan readily.⁵⁴ Immediate tanning is most conspicuous in subjects who already have some degree of either constitutive or facultative pigmentation, in whom it can be sufficient to complicate the visual assessment of experimental UVA-radiation-induced erythema even after 24 hr.

Immediate tanning appears not to represent an increase in epidermal melanin or melanocytes, but is an alteration in melanin already present in the epidermis. Several changes have been noted. Parts of the melanin molecule are oxidized to semiquinone-like free radicals.⁵⁵ This distribution of melanosomes is altered; they move from a perinuclear position in human melanocytes, peripherally into the dendrites, and are transferred into keratinocytes. The distribution of microfilaments and microtubules in the melanocyte also is altered, which suggests that these elements may be involved in the rapid peripheral movement of melanosomes.⁵⁶

The function of immediate tanning is not clear. A protective effect of immediate tanning against UVB-radiation-induced erythema has not been demonstrated¹³; however, this finding by no means precludes the possibility that it protects against other biologic responses.

5.5.2. Delayed Tanning

Delayed tanning, which, in contrast to immediate tanning, is clearly photoprotective, first becomes visible about 72 hr after irradiation and reflects an increase in epidermal melanin content. The major action spectrum for delayed tanning resembles that for erythema, lying in the UVB spectrum (Figure 5.1), but UVC, UVA, and visible radiation can also cause delayed tanning. Relative dosage thresholds for erythema and delayed tanning differ with wavelength. In genetically capable persons, suberythemal doses of UVA radiation can be melanogenic, whereas erythemogenic doses of UVB radiation are required. This difference is particularly noticeable after repeated doses; 0.5 MED of UVA radiation repeated daily for 9 days induces marked pigmentation without erythema, whereas erythemogenic doses of UVB radiation are required for pigmentation to occur.⁵⁷ In very fair-skinned individuals, however, the action spectrum for delayed tanning and erythema are identical at wavelengths between 313 and 405 nm.⁵⁸ These subjects show a marked dissociation of the two action spectra at wavelengths less than 296 nm, where melanogenesis may require 2 to 8 MED. This dissimilarity can be rationalized in terms of skin optics; significant absorption of shorter wavelengths by proteins and nucleic acids in the upper layers of the epidermis could lead to erythema, while limiting the degree of exposure to which the melanocytes, situated in the basal layer, are subjected. Another consideration is that the results of this study apply to fair-skinned individuals in whom erythema is readily induced, but who are genetically or constitutionally poor tanners.

Delayed tanning is associated with an increased melanocyte population as well as increased melanocyte activity. In general, single exposures stimulate functional activity, and repeated exposures increase melanocyte numbers as well. This increase in population may result from recruitment of dormant melanocytes and from mitosis. Mice irradiated repeatedly with UVB radiation showed a four- to sixfold increase in melanocyte population in 11 days; the melanocyte population reverted to normal levels a few months after irradiation ceased.^{59,60} A smaller increase in melanocytes was also observed in nonirradiated sites, suggesting that a systemic factor might be involved. In man, multiple exposures to UVB radiation are required for a demonstrable increase in melanocyte population to occur.⁶¹ After irradiation, the dendrites grow and branch, and tyrosinase activity is enhanced. The melanocytes grow and proliferate, and transfer to keratinocytes more rapidly, resulting in a great increase in the number of melanin granules in the epidermis.⁶¹

The main function of delayed tanning is photoprotection, although the protection conferred by melanin alone without alterations in the stratum corneum is, at best, moderate. For example, Caucasians who had a deep UVA-radiation-induced tan showed only a two- to threefold increase in MED of solar-stimulating radiation.⁶² Little or no change was seen in the thickness of the stratum corneum, although transmission of UVB radiation was halved in the tanned isolated stratum corneum, presumably as a result of increased melanization. In contrast, the increase in MED is greater when tanning is induced by repeated UVB irradiation, for example, during psoriasis phototherapy,¹¹ because of the associated increase in thickness of the epidermis and stratum corneum. In vitiligo, substantial photoprotection after exposure to UV radiation results from thickening of the stratum corneum, which occurs more in the depigmented skin than in the surrounding normal skin.⁶³ Thus, in light-skinned individuals, skin thickening is probably the more important photoprotective response, whereas in dark-skinned individuals, pigmentation is the more important response.

Because of its complex molecular structure, melanin may also protect against UV-radiation-induced damage by interacting with toxic photoproducts and by acting as a free radical absorber, in addition to its direct photon-absorbing capacity.⁵⁵

5.6. Changes in the Kinetics of Epidermal Cells

Ultraviolet radiation causes the epidermis to thicken, thereby increasing the tolerance of the skin to subsequent radiation. Studies in humans have shown that, after a single exposure to UVB radiation, the stratum spinosum thickens twofold and the stratum corneum thickens 1.5 to threefold within 1 to 3 wk.⁶⁴ This response is the result of a sustained increase in epidermal mitosis that

occurs after a single exposure and is associated with similar changes in rates of DNA, RNA, and protein synthesis. Preceding these responses, there is a transient depression of mitosis and macromolecular synthetic activity.

Most studies of these responses have used single doses of UVB radiation in human or animal skin.⁶⁵⁻⁶⁸ Macromolecular synthesis is greatly reduced 1 to 6 hr after irradiation, but is most stimulated 24 to 48 hr after irradiation. Changes in mitosis follow a similar time course. When nuclear labeling with tritiated thymidine is examined, the early depression of heavy labeling (indicating cells passing through the S phase of the cell cycle) is preceded by a considerable increase in the number of cells showing sparse labeling, indicating DNA repair.⁶⁶ The phase of DNA repair peaks immediately after irradiation and is diminished in 5 hr. The stimulation of macromolecular synthesis and mitosis that follows persists for several days and may still be observed after 1 wk. This sustained increase results in the thickening of epidermis and stratum corneum and is most marked after exposure to UVC and UVB radiation, but it has also been observed following suberythemal doses of UVA radiation.¹³

The pathway through which stimulation of epidermal proliferation is mediated is not known. The observation that UV radiation induces a large increase in epidermal ornithine decarboxylase provides some interesting clues about possible mechanisms of stimulation. Ornithine decarboxylase is the rate-limiting enzyme in the synthesis of the polyamines, putrescine, spermidine, and spermine, which are found in most normal tissues and which are increased in amount in proliferative states.⁶⁹ In mice, the levels of ornithine decarboxylase begin to rise 2 hr after irradiation, reach a small plateau at 4 hr and a more sustained plateau at 24 to 30 hr, when levels may be two hundredfold greater than initial levels: ornithine decarboxylase declines to normal levels after 48 to 72 hr.^{70,71} UVB and UVC radiation both induce the enzyme at doses similar to those causing erythema; induction by UVA radiation has not been demonstrated except in the presence of the photosensitizers anthracene and 8-methoxysoralen.⁷² A second enzyme in the polyamine biosynthetic pathway, S-adenosyl methionine decarboxylase, is also induced by UV radiation.

The significance of UV-radiation-induced polyamine biosynthetic activity is not certain. Increased polyamine biosynthesis precedes and may be related to the wave of epidermal proliferation that follows radiation. Putrescine causes DNA synthesis to increase 2.5 times in mouse skin in which endogenous polyamine biosynthesis has been inhibited by topical steroids.⁷³ However, the administration of α -difluoromethyl ornithine, a potent inhibitor of ornithine decarboxylase, to UVC-irradiated mice, has been shown to substantially reduce polyamine synthesis without affecting DNA synthesis.⁷⁴ Tumor-promoting compounds such as phorbol esters also induce ornithine decarboxylase, but with a somewhat more rapid time course⁷⁵; a similar time course is seen when repeated exposures of UVB radiation are given.⁷⁶ The application of tumor promoters, like UV radiation, is followed by a wave of epidermal proliferation and thickening.⁷⁷

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Chapter 6

SALT: Skin-Associated Lymphoid Tissues

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6.1. Introduction

Few tissues or organ systems are as complex as the immune system, except perhaps the nervous system. Eighty years ago, when the immune response was first described, investigators naively thought that the immune response of an organism to exogenous antigen was a unitary, if mysterious, process that equipped the individual to nullify once and forever the pathogenic potential of specific invading microorganisms. Now, as we proceed through the second “Golden Age of Immunology,” we know that the immune response is heterogeneous and that it is comprised of effector mechanisms that are both molecular and cellular. Moreover, we know that specific immune responses to exogenous antigens are associated with specific tissues and organs. Thus, antigens that gain access to the body through the oral route confront the immunologic apparatus initially through the gut and elicit a relatively unique immune response at mucous membrane surfaces of the gut and perhaps of the respiratory tract. In fact, immune responses elicited by antigens within one tissue may be deleterious to the host when these antigens are encountered at or through a different, unrelated tissue or organ. Thus, the immune system appears to be comprised of subsystems, regional spheres of influence, in which a specific tissue, its constituent (sessile) cells, and complementary immunocompetent (mobile) lymphocytes are integrated into an internally consistent response to antigen. Blood-borne effector cells generated in these regional spheres can circulate to other tissues and express themselves inappropriately in the new territory. In this chapter, we will discuss the unique regional sphere of immunologic influence of the skin, which is comprised of sessile cells, mobile cells, and regional draining lymph nodes that have been designated as *skin-associated lymphoid tissues* (SALT).

Certain types of cells and anatomic structures are known to be essential for the induction and elicitation of an immune response. These factors transcend

any regional differences in immune reactivities initiated through different types of tissues and surfaces. Upon exposure to exogenous antigen, the first step in the immune response is uptake of antigen by cells capable of processing and/or otherwise focusing the antigen for effective presentation to immunocompetent lymphocytes. The precise identity of the antigen-presenting cells is controversial; both macrophages harvested from the spleen and from the peritoneal cavity¹ and bone-marrow-derived cells, termed *dendritic cells*,² have been shown to present antigens. Some investigators classify dendritic cells as a subpopulation of macrophages; however, this seems unlikely in light of our present knowledge. All antigen-presenting cells studied to date express on their surfaces the products of genes located within the major histocompatibility complex of all mammals, class II genes (murine I region of H-2, human HLA-DR region). Antigen may be recognized by T lymphocytes in association with class II gene products on the surface of antigen-presenting cells. The process of associative recognition is probably also essential (directly or indirectly) for the perception of antigen by B lymphocytes. T and B lymphocytes are activated specifically through antigen recognition aided by the local secretion of interleukin-1, a cytokine produced by antigen-presenting cells that acts on responding T lymphocytes.³ As a consequence, clonal proliferation and differentiation of T and B lymphocytes is initiated. These events are usually considered to take place within the cortical region of lymph nodes and the equivalent region of the splenic periarteriolar sheath. The cellular and molecular consequences of T cell activation (lymphokines, cytotoxic cells, cells that mediate delayed-type hypersensitivity, and cells that regulate the immune response) and of B cell activation (specific antibodies, memory B cells, regulating anti-antibodies) then disseminate systemically through the lymphatics and blood.

There are many factors that determine the specific destination of these cells. Certain lymphocytes fail to distribute themselves randomly throughout somatic tissues. de Sousa⁴ first proposed the term, *ecotaxis*, to describe the capacities of distinct cell populations to migrate and localize within separate microenvironments of the various peripheral lymphoid organs. Lymphocytes that have encountered antigen initially within the alimentary tract demonstrate a proclivity for recirculating thereafter between gut and the systemic vasculature.⁵ The concept of gut-associated lymphoid tissues (GALT) was developed from these observations.⁶ Other investigators proposed that putatively unique circuits of lymphocytes also existed for the respiratory tract, (bronchial-associated lymphoid tissue or BALT),⁷ the breast, (mammary-associated lymphoid tissue or MALT),⁸ and the external eye, (conjunctival-associated lymphoid tissue or CALT).⁹ Evidence reported in this chapter suggests the existence of a circuit of unique lymphocytes that display special tropism for skin.¹⁰ We propose that SALT comprises a unique set of antigen-presenting cells localized within the skin, a corresponding set of recirculating T and B lymphocytes, and an integrating set of draining lymph nodes with a microenvironment that is

complementary to a unique cutaneous environment. Together, these interacting cells and tissues provide skin with an essential and unique capacity to deal with antigenic challenges that occur at the cutaneous surface.

6.2. Skin as a Microenvironment for Antigen Presentation

During development of an immune response, effective presentation of antigen is required at two critical stages: (1) During induction, when antigen must be presented initially to immunocompetent but naive lymphocytes, and (2) during expression, when antigen serves as the specific target of the cellular and antibody effector modalities. The skin is a very effective site for antigen presentation at the effector phase. The classical tuberculin skin reaction, which has been used often as a model for studying the expression of antigen-specific immune responses requires successful antigen presentation. However, this type of antigen presentation is less demanding than the induction of immune responses; the induction of an immune response to antigen requires significantly more stringent conditions. It is here that the concept of SALT is severely tested: Does the skin afford an environment in which antigen can be presented effectively to T and B lymphocytes, that is, can these events be accomplished completely, or largely so, within the skin itself?

Brent et al.¹¹ in studies on the cellular basis of alloimmune responses found evidence that supports the hypothesis that antigen presentation at induction of immune responsiveness can take place within the skin. These investigators examined the extent to which immunity to transplantation alloantigens could be assessed *in vivo* through manipulation of lymphocytes sensitized to these specificities. When lymphoid cells from alloimmune guinea pigs were placed intradermally into recipient animals expressing the relevant alloantigens, delayed inflammatory reactions occurred that closely resembled reactions to tuberculin antigens in tuberculin-positive animals. During the course of these studies, these workers noticed that lymphocytes from normal, unsensitized donors, used ostensibly as controls, frequently evoked similar, although less intense, positive delayed skin reactions. Experiments in which appropriate F1 hybrid donors and/or recipients were used showed that the inflammatory reactions incited by normal (unsensitized) lymphoid cells after their inoculation into the skin of allogeneic (and similarly unsensitized) recipients resulted from the *in situ* recognition of alloantigens on the part of the injected cells.¹² The inflammatory reaction resulted from the activation of these antigen-reactive cells into effectors of delayed-type hypersensitivity reactions. Thus, within a single inoculation site in the skin, an entire immune process had occurred, from antigen presentation through antigen recognition, activation, and then elicitation. Although responses in distant sites, such as draining lymph node and spleen, may have contributed

to the intensity of the response, the entire sequence had taken place in situ.

Ramseier and Strelein¹³ further investigated in situ alloimmunization and expression in the skin. They discovered that lethal irradiation of Syrian hamsters robbed them of the ability to mount responses to intracutaneously injected alloantigenic cells. These immunoincompetent animals, however, retained the ability to display inflammatory reactions incited within their skin by inoculations of mixtures of histoincompatible lymphocytes from immunogenetically disparate hamsters. In fact, the skin of these irradiated hamsters even responded to the inoculation of allogeneic lymphoid cell mixtures from rats and mice by developing intense inflammatory reactions. These and related experiments confirmed that skin contains the cells and anatomic structures necessary for T lymphocytes to perceive alloantigenic cells, to become activated in situ, and to instigate an inflammatory reaction that is clinically and histologically indistinguishable from conventional cell-mediated immune responses.

Skin serves as a microenvironment not only for presentation of alloantigens, but also for the induction of contact hypersensitivity.^{14,15} In a series of elaborate and difficult experiments, Macher and Chase^{14,15} used radiolabeled hapten to study contact hypersensitivity reactions in rodents. The ears of the rodents were injected with radiolabeled hapten, and the injected skin was excised after various lengths of time. Analysis of the site showed that although most of the hapten never bound to cutaneous proteins and was swept away by the flow of afferent lymph, a small, but extremely important, amount of hapten remained at the injected site. This residue of hapten, which was bound as a derivatization product to skin proteins, was found to be essential for the induction of contact hypersensitivity. Macher and Chase found that if the injected ear skin was excised within 24 hr of painting, sensitization did not occur. They reasoned that this length of time was probably required for lymphocytes moving through the skin to recognize hapten and thereby to become activated. In these experiments, excision of the draining lymph node also prevented successful induction of contact hypersensitivity, a finding that suggested that both the site of derivatization and the draining lymph node were required for the sensitization process. Macher and Chase proposed that the early phase of induction, which they termed peripheral sensitization, actually took place within the skin treated with hapten; the subsequent involvement of the lymph node was viewed as a means of amplifying the response already initiated within the skin itself.^{14,15} The term *peripheral sensitization*, frequent during the 1960s, has fallen into disuse over the past decade. Yet, in the more than a decade since the experiments just described were completed, no evidence has been advanced to suggest that the results were invalid. The concept of peripheral sensitization deserves to be re-examined in light of contemporary standards. In any case, the assertion that lymphocytes can be activated by antigen in the cutaneous milieu seems to be valid.

One contemporary insight that has transformed our understanding of the antigen-presenting potential of skin is derived from recent studies on epidermal

Langerhans cells. The original association between Langerhans cells and immune reactions in the skin was based on the study of expression of cutaneous contact hypersensitivity reactions.¹⁶ This demonstration of Langerhans cell-lymphocyte interactions was so dramatic that it catalyzed a vigorous study of Langerhans cells in terms of immunologic functions. A more complete discussion of the large body of recent information on Langerhans cells appears in Chapter 9; only pertinent aspects will be restated here. Langerhans cells are bone-marrow-derived, mesenchymal cells¹⁶ that migrate slowly through the epidermis and share certain important properties with macrophages: Cell surface Fc receptors, C3b receptors, and Ia alloantigens.¹⁷⁻²⁰ However, unlike macrophages, they do not usually conduct phagocytosis. They may be closely related to dendritic cells, which are similarly derived and which also process and present antigens to T lymphocytes. Numerous studies conducted *in vitro* attest to the extraordinary capacity of Langerhans cells to present a wide variety of antigens, including alloantigens, to T lymphocytes.²¹ *In vivo* evidence in support of a role in antigen presentation for Langerhans cells rests largely on a circumstantial argument: Cutaneous sites that have been artificially depleted of, or are naturally deficient in, epidermal Langerhans cells are unable to support the induction of contact hypersensitivity to simple haptens.²² This line of investigation is the contemporary descendant of the observations of Macher and Chase and reaffirms that the cellular basis for antigen presentation resides among constitutive epidermal cells.

In an effort to prove that Langerhans cells are the essential effector cells of contact hypersensitivity reactions in the skin, attempts have been made to isolate and purify Langerhans cells. Two groups of investigators are working to isolate (or enrich cell suspensions for) Langerhans cells, derivatize them with hapten, and demonstrate that derivatized Langerhans cells (alone, but not with derivatized keratinocytes), when injected intravenously, are able to evoke contact hypersensitivity, rather than unresponsiveness. As might be expected from premature experiments using poorly defined, "Langerhans-cell-enriched" epidermal cell suspensions, the results of the two groups differ.^{23,24} Although both groups are working on the idea that Langerhans cells are the critical antigen-presenting cells of the epidermis, the theories of Ptak et al.²³ are widely quoted. Ptak's group claims that hapten-derivatized Langerhans-cell-enriched epidermal cells sensitizes skin to hapten when injected intravenously. New techniques for isolation and purification of Langerhans cells are being developed and should make it possible for these investigators to determine definitively whether Langerhans cells are the antigen-presenting cells in question.

The process of antigen presentation to lymphocytes by macrophages is associated with the production of soluble mediators by the macrophage. The best-defined of these mediators, interleukin-1,³ has the capacity to activate T lymphocytes that are simultaneously confronted with antigen and to cause these cells to secrete interleukin-2.²⁵ Recently, Luger et al.²⁶ and Sauder et al.²⁷ examined the possibility that interleukin-1-like substances can be produced by

cells within the epidermis. The former used a transformed keratinocyte cell line, PAM 212, and the latter used nontransformed “normal” keratinocytes as sources of a factor that activates thymocytes, the epidermal cell-derived thymocyte-activating factor (ETAF). The similarities between this factor and interleukin-1 are striking. Both interleukin-1 and ETAF increase proliferation of thymocytes, either alone or in the presence of suboptimal concentrations of mitogens. Neither interleukin-1 nor ETAF alone can increase proliferation of mature peripheral T cells; however, both can augment the production of interleukin-2 by stimulated, mature peripheral T cells. Since identifiable Langerhans cells were not present in the keratinocyte cultures employed by either Luger or Sauder and co-workers,^{26,27} ETAF was almost certainly produced by the keratinocytes themselves. These findings raise the interesting possibility that keratinocytes and Langerhans cells each can contribute to the process of presenting the appropriate signals to immunocompetent T cells required for such cells to respond optimally to antigenic signals. Again, because preparations of Langerhans cells are not available, investigators as yet have not determined whether these cells are also capable of producing interleukin-1-like mediators.

Nonetheless, the studies summarized in this section give a strong circumstantial support to the hypothesis that a special microenvironment exists within skin, perhaps even within the epidermis, for the effective presentation of antigen to circulating, mature lymphocytes. Not only can mature lymphocytes placed in an allogeneic cutaneous environment establish an alloimmune inflammatory reaction *in situ*, but Langerhans cells have been identified and characterized as antigen-presenting cells, and factors have been obtained from epidermal cells with the property of activating lymphocytes in the presence of antigen.

6.3. Affinity between T Lymphocyte Subsets and the Skin

6.3.1. Preferential Migration of Malignant T Cells to Skin

Perhaps the most striking clinical evidence that there is a special relationship between lymphoid cells and the skin comes from an analysis of the clinical, histopathologic, and immunologic characteristics of lymphoid malignancies that involve the skin. This analysis, in turn, depends upon an understanding of two general concepts of contemporary cellular immunology. First, the immune system is comprised of a complex network of interacting cell subpopulations, each of which has not only a discrete and limited functional repertoire, but also unique phenotypic characteristics, such as cell surface antigens and/or membrane receptors. Secondly, malignancies of cells of the lymphoreticular system involve the unrestrained expansion of populations of abnormal cells

that, to a greater or lesser degree, retain at least some of the functional activities and phenotypic markers of the normal cell from which the neoplasm presumably arose. Thus, the development of increasingly sophisticated immunologic techniques has made it possible to determine whether human lymphoreticular neoplasms are derived from T-cell, B-cell or macrophage/monocyte lineage, and to determine whether the malignant cells are derived from a phenotypically and functionally discrete subpopulation of cells contained within one of these normal major classes.

The most common lymphocyte malignancies which are characterized by malignant cutaneous infiltrates originate from T cells. The generic term *cutaneous T-cell lymphoma* (CTCL) has been proposed to cover this group of diseases, which may, in fact, have differing clinical and histopathologic presentations.²⁸ Cutaneous T-cell lymphomas include not only classic mycosis fungoides (MF) and its leukemic, erythrodermic variant, Sezary syndrome (SS), but also many cases of non-Hodgkin's lymphoma-leukemia, which may involve the skin and which, on morphologic criteria, have been called variously reticulum cell sarcoma, immunoblastic sarcoma, histiocytic lymphoma, diffuse, poorly differentiated, lymphocytic lymphoma, among others. In contrast to the frequently widespread, and perhaps primary, involvement of the skin seen in the MF-SS variants of CTCL, non-T-cell lymphoma-leukemias more rarely give rise to specific skin lesions, and when they do, it is usually late in the natural evolution of the malignant process, i.e., the skin lesions appear after the disease has been confirmed in extracutaneous sites. Furthermore, examination of the frequency of malignant cutaneous infiltrates in patients with non-Hodgkin's lymphoma-leukemia other than MF-SS has also provided evidence that malignancies of T lymphocytes more frequently involve the skin than non-T-cell malignancies. Yamanaka and co-workers²⁹ recently described the results of cell surface marker analysis of 44 cases of non-Hodgkin's lymphoma other than MF-SS. In the 31 cases of non-T-cell disease, specific skin lesions at any time during the course of the disease were noted in only 6% of the cases. Alternatively, malignant cutaneous infiltrates were observed at some time in 6 of the 13 (46%) patients whose malignant cells had the surface markers of T lymphocytes.

The histopathologic pattern of cutaneous involvement seen in non-T-cell lymphoma-leukemia is strikingly different from that seen in the MF-SS variants of CTCL. The most consistent finding in non-T-cell malignancies is the presence of a tumor-free grenz zone in the most superficial dermis, with either a diffuse or patchy infiltrate of malignant cells in the reticular dermis and/or subcutaneous tissue. The finding of clearly malignant cells within the epidermis in areas other than those in which there has been obvious necrosis of the overlying epidermis with secondary extension of the tumor cell mass into this necrotic epidermis is extremely uncommon.³⁰ The one relevant exception to this generalization is the special and extremely rare case of histiocytosis X, in which the malignant cells have been shown to bear membrane markers characteristic of

macrophages,³¹ and intracytoplasmic inclusions^{32,33} identical to those seen in Langerhans cells. Very characteristically, the cutaneous infiltrates in MF–SS do not spare the papillary dermis, i.e., there is no grenz zone. Furthermore, epidermal invasion by atypical hyperchromatic, hyperconvoluted T cells, either as single cells or as clusters (Pautrier microabscesses), is particularly characteristic. This phenomenon of the malignant T cells showing a particular affinity for the epidermis is termed *epidermotropism*, and is one of the most identifiable histopathologic characteristics of these diseases.³⁴

Epidermotropism is not a characteristic feature of non-T-cell malignant cutaneous infiltrates, nor is it an invariable feature of all malignant T-cell infiltrates. Recently T-cell chronic lymphocytic leukemia (CLL) has been described.^{35,36} The frequency of specific cutaneous infiltrates (that have presented clinically either as generalized erythroderma or as more discrete nodules and tumors) has been nearly 50% in the patients reported to date. However, in contrast to the typical epidermotropic pattern of MF–SS, the histopathology in T-cell CLL has revealed involvement of the middle and lower dermis and subcutaneous tissue, with a striking grenz zone and no infiltration of malignant cells into the epidermis. In this regard, T-cell CLL cutaneous histopathology resembles that seen with other non-T-cell malignant cutaneous infiltrates. Edelson³⁷ has recently proposed that MF and SS be termed epidermotropic variants of CTCL in order to contrast these diseases with other forms of CTCL (nonepidermotropic variants of CTCL).

Considerable data have accumulated to suggest that the epidermotropic variants of CTCL are derived from a functionally and phenotypically discrete subpopulation of T cells and that the nonepidermotropic variant, T-cell CLL, is derived from another subpopulation of T cells. Studies over the past five years have shown that in approximately 40% of the cases examined, the malignant T cells in the blood of patients with SS³⁸ and those in the extracutaneous tumors and involved lymph nodes of patients with MF³⁹ can act as pure populations of helper-inducer cells, i.e., they can augment significantly the production of antibody by mitogen-stimulated normal B cells. Very recently, monoclonal antibodies have been prepared that identify antigens expressed on the two major functionally discrete subpopulations of human peripheral T cells (reviewed by Haynes⁴⁰). OKT-4 is an antigen expressed on approximately 60% of peripheral T cells; this determinant identifies cells which functionally behave as helper-inducer cells. Alternatively, OKT-8 and OKT-5 are antigens expressed on the approximately 30% of peripheral T cells that act as suppressor or cytotoxic cells. The malignant T cells in MF–SS have the cell surface phenotype (e.g., OKT-4⁺) that has clearly identified them as being derived from T cells of the helper-inducer lineage.⁴¹ In contrast, the nonepidermotropic CTCL variant, T-cell CLL, has the functional phenotype of cells belonging to the suppressor-cytotoxic lineage.⁴² In all the cases, when a regulatory activity could be found (3 of 6 cases), the activity was invariably suppression of mitogen-

induced B-cell immunoglobulin production instead of helper function. Furthermore, the cell surface phenotype of the malignant cells in some cases of T-cell CLL was OKT-8⁺.⁴³ Thus, cutaneous involvement is more commonly seen with T-cell neoplasms than with non-T-cell neoplasms. In addition, cell surface marker phenotyping shows that malignant T cells that display a tendency to move into the epidermis at sites of cutaneous infiltration almost always belong to a discrete helper-inducer cell subpopulation.

6.3.2. Immunocompetent T Cells in Normal Skin

The presence of lymphocytes within the epidermis of normal human and rat skin was initially reported by Andrew and Andrew.⁴⁴ These investigators, looking at routine histologic sections by light microscopy, found that lymphoid cells constituted 1 to 4% of the cells in the basal layer of the epidermis. More recently, Lemmel and Fichtelius,⁴⁵ in an examination of normal guinea pig skin, also noted the presence of significant numbers of lymphoid cells within the epidermis. Although electron microscopy and immunologic phenotyping of these cells were not used in these studies, the results strongly suggest, but do not prove, that lymphocytes are present, not only in the dermis of the skin, but within the normal epidermis. Furthermore, small but significant numbers of lymphocytes are contained in the afferent lymphatics draining from normal skin to the peripheral lymph nodes.⁴⁶⁻⁴⁸ The cell surface phenotype of these lymphocytes has also been examined. In sheep, only 5 to 10% of the lymphocytes in afferent lymph draining the popliteal lymph nodes were B lymphocytes.⁴⁹ T lymphocytes also predominate in human peripheral lymph.⁵⁰ A significant proportion of the cells in afferent lymph draining the skin consists of so-called veiled cells, interdigitating reticular cells that appear to be cells related if not identical to macrophage/monocytes; in addition, the presence of cells with the characteristic Birbeck granules, identifying them as Langerhans cells, have also been observed in normal peripheral afferent lymph.⁵¹

Several investigators have suggested that the T cells in normal skin are immunocompetent. Solomon⁵² grafted skin of the parent genotype to a semi-allogeneic F₁ hybrid recipient chicken and observed that the graft was rejected. Since, according to conventional transplantation immunology, the F₁ recipient should be genetically incapable of mounting an effective host-versus-graft response against the parent strain histocompatibility antigens, he concluded that a graft-versus-host reaction initiated by cells within the donor graft must have caused the rejection. Reasoning that since newly hatched chicks are immunologically incompetent, cells of hematogeneous or lymphoid origin present within skin would be similarly immunoincompetent and incapable of mounting a graft-versus-host response, Solomon then grafted newly hatched chick skin of the parent genotype on adult semiallogeneic F₁ recipients. The grafts survived indefinitely, presumably because the putative passenger cells were unable to

recognize histoincompatibility in the host. Solomon hypothesized that adult chicken skin contained a population of immunocompetent cells that was able, when confronted by histocompatibility antigens in the recipient, to act against them. In the skin graft bed, the net effect of this confrontation was to elicit a sufficiently intense inflammatory reaction that, although similar to graft-versus-host, was ultimately detrimental to the graft survival.

More recently, Barker and Billingham⁵³ utilized local lymph node hypertrophy in response to grafting with allogeneic skin in mice to examine further the question of whether cells with immunocompetence are present in a graft of murine skin. When an (A × B)F₁ hybrid was grafted with the skin from another identical hybrid donor, essentially no enlargement of the draining axillary lymph node was observed. Alternatively, when an F₁ hybrid was grafted with skin from one of the parent strains, there was significant swelling of the draining lymph node. Thus, the hypertrophy of the node draining the bed of the parent strain skin graft must have resulted from the confrontation within that node of passenger lymphocytes transferred within the graft, that is, cells that leave the graft to enter the draining node and there elicit a local graft-versus-host reaction. Since it is known that graft-versus-host reactions are initiated by T lymphocytes, both of these experiments strongly suggest that immunocompetent T cells are present in normal skin. However, neither of these studies^{52,53} showed conclusively that the immunocompetent T cells present in the skin grafts were within the substance of the skin itself and not simply trapped within the vascular bed. By using antibodies that can discriminate between subpopulations of lymphocytes and newly developed immunofluorescent or immunoperoxidase-staining techniques for *in situ* identification of cells bearing distinctive cell surface markers, investigators should be able to determine the localization and phenotype of lymphoid cells in the clinically normal skin from both humans and laboratory animals.

6.3.3. Immunocompetent T Cells in Benign Inflammatory States

In situ phenotypic identification of lymphocytes in human inflammatory dermatoses, such as lichen planus, has indicated that most infiltrating cells are T lymphocytes.^{54,55} Very recently Haynes and co-workers⁵⁶ have utilized immunofluorescence to characterize the subpopulation phenotype of lymphoid cells infiltrating various benign inflammatory skin lesions in man, including sites of allergic contact dermatitis and delayed-type hypersensitivity. The OKT-4⁺ (helper-inducer) subpopulation accounted for most T cells infiltrating the dermis and the epidermis in these inflammatory sites; OKT-8⁺ (suppressor-cytotoxic) T cells were present in substantially smaller numbers. Whether there is a selective infiltration of helper-inducer T cells and/or whether the quantitative composition of cutaneous infiltrates in both the dermis and epidermis

is proportionally identical to the subpopulation composition of peripheral blood (the immediate source of the cells found within the skin) has not yet been determined.

There is a considerable amount of data on the relative capacities of different lymphoid cell populations to move from the blood into cutaneous inflammatory sites in experimental animals. Most studies have used intravenously injected indicator cells that have been radiolabeled so that their accumulation in ongoing inflammatory reactions in the skin and in other organs and tissues can be quantitated. Several investigators have examined the migratory properties of cells obtained either from the peripheral lymph nodes (PLN) of normal animals or from the PLN draining the sites of epicutaneous application of contact-sensitizing agents such as oxazolong (Ox) or dinitrofluorobenzene (DNFB). After in vitro labeling with either ^{51}Cr or [^{125}I] iododeoxyuridine, these cells were injected into mice whose ears were undergoing either contact hypersensitivity or primary irritant dermatitis reaction to some chemically unrelated agent. The proportion of radiolabeled cells nonspecifically recruited from the blood into these cutaneous reaction sites was determined by counting the radioactivity present in the ears 24 hr later. Radiolabeled PLN cells stimulated with antigens such as Ox, which are known to cause lymphoblast transformation, proliferation, and differentiation of both T and non-T cells, localized in the inflamed skin sites significantly more often than cells obtained from unstimulated nodes.⁵⁷ Utilizing velocity sedimentation to obtain large cells (principally immunoblasts) and small cells from stimulated PLN, these investigators⁵⁸ also found that recently stimulated cells (immunoblasts) entered either irritant or contact hypersensitivity reaction sites significantly more often than unstimulated cells did. Similarly, many more lymphoblasts than small lymphocytes entered other sites of cutaneous inflammation, such as skin allografts⁵⁹ and sites of delayed-type hypersensitivity reactions.^{60,61} Asherson and co-workers⁵⁸ found that T-cell-depleted suspensions from stimulated PLN had a markedly decreased capacity to be nonspecifically recruited into contact dermatitis sites; Bhan et al.⁶² found that normal spleen T cells were markedly superior to spleen B cells in their capacity to emigrate into sites of skin allografts. These studies show that T cells, especially those that have undergone recent stimulation and/or division in peripheral lymphoid tissues, have a significantly greater affinity for sites of cutaneous inflammation than non-T cells derived from these same tissues.

These studies only provided data about the relative capacities of various indicator populations to enter the overall site of cutaneous inflammation and did not address the issue of whether there is selective emigration of a subpopulation of normal lymphoid cells from the blood into the dermis and then into the epidermis at such inflammatory sites. Liden⁶³ employed the technique of autoradiography to study tissue sections of contact hypersensitivity reaction sites in guinea pigs. When immune animals had received several subcutaneous injec-

tions of [³H]thymidine (used to label selectively the dividing cells in the draining peripheral lymph nodes) immediately before elicitation of a contact hypersensitivity reaction, significant numbers of labeled cells were found in both the dermis and epidermis.⁶³ The ratio of labeled to unlabeled lymphoid cells in the epidermis was significantly greater than the ratio in the dermis. These results, in addition to demonstrating that recently divided lymphocytes can emigrate into the epidermis at sites of contact hypersensitivity reactions, also suggest that there may be selective exocytosis of recently divided lymphocytes in the epidermis. In other studies in which [³H]thymidine was used to label selectively cells synthesizing DNA in the bone marrow (a site known to contain relatively few mature T cells), labeled cells were rarely observed in the epidermis, although substantial numbers of labeled cells were observed in the dermis at contact hypersensitivity reaction sites.⁶⁴ Furthermore, Bhan et al.,⁶² in their studies of the capacity of T-cell-enriched versus B-cell-enriched populations to emigrate into mouse skin allografts, found small numbers of labeled T cells in the allograft epidermis, but no labeled B cells within the epidermis. These two studies suggest that T cells are more epidermotropic than non-T cells.

Recent studies in our laboratory have been directed at the quantitative comparison of the relative capacities of various populations of lymphoid cells to be recruited nonspecifically from the blood into the dermis and the epidermis in the ears of mice undergoing either primary irritant or contact hypersensitivity reactions. We radiolabeled various preparations of lymphoid cell suspensions in vitro with [³H]uridine and injected the radiolabeled cells intravenously into normal or immune syngeneic mice. The ears of the mice were then challenged with DNFB, were removed 24 hr later, and were processed for autoradiographic analysis. The indicator populations included normal PLN cells, PLN cells from mice sensitized with the noncrossreacting antigen Ox, (both populations were shown to contain approximately 70% T cells), T-cell-depleted Ox-stimulated PLN cells (less than 12% T cells), and normal bone marrow cells (less than 12% T cells). The results of these studies can be summarized as follows: (1) Recently stimulated PLN cells not only have a significantly greater capacity to leave the blood to enter the dermis at sites of contact hypersensitivity reactions than unstimulated PLN cells, but once in the dermis of the reaction site, the recently stimulated cells are significantly more likely to move into the epidermis and be retained there. (2) Bone marrow cells, a population rich in recently divided cells but relatively poor in T cells, enter the dermis from the blood stream more readily than Ox-stimulated PLN cells. However, in spite of being present in the overall site in smaller numbers, the numbers of labeled Ox-stimulated PLN cells per cross section in the epidermis was about 4 times the number of labeled bone marrow cells per cross section in the epidermis. (3) T-cell-rich, Ox-stimulated PLN cells were not only significantly more able than the T-cell-depleted, Ox-stimulated PLN cells to enter the dermis at the reaction site, but once in the dermis they were 3 times as likely to move into the epidermis and

to be retained there. Thus, different populations of normal lymphoid cells have very different capacities to be nonspecifically recruited from the blood into the dermis of contact hypersensitivity reactions in mice. Once in the dermis of the reaction site, these different populations of cells also display strikingly different capacities to move into the epidermis. The characteristics which favor epidermotropism of lymphocytes include the presence of T-cell markers and recent stimulation or division of these cells. These observations are consistent with the hypothesis that epidermotropism is a property characteristic of a subpopulation of T lymphocytes. Additional studies are directed at the more complete characterization of the phenotype of cells that exhibit this migration pattern within the skin.

6.3.4. Factors That Influence Localization of T Lymphocytes within Specific Tissues

6.3.4.1. Blood Flow

Recently, Ottoway and Parrott⁶⁵ measured the simultaneous changes in the blood flow and cell traffic after topical application of Ox to the ears of nonimmune mice. The increased blood flow into the ears was directly proportional to the increase in the migration of lymphoblasts to the skin. A much smaller increase in the migration of small lymphocytes to the cutaneous inflammatory site was noted; however, this emigration was not proportional to the increase in blood flow. Strikingly different patterns of migration were seen in the draining lymph nodes. Although there was also a marked increase in blood flow to the draining nodes, in this instance, the localization of small lymphocytes was correlated with the increased blood flow. This correlation was not seen with immunoblasts. These results suggest that although increased blood flow is an important factor in increasing the localization of lymphoid cells at a given site, other local and presumably tissue-specific factors control which subpopulations of lymphocytes will show an enhanced capacity to leave the blood at that site.

6.3.4.2. Vascular Permeability

Increase in vascular permeability with subsequent leakage of serum proteins and edema occurs in various cutaneous inflammatory reactions, including both contact hypersensitivity and primary irritant reactions. It has been postulated that this increase in vascular permeability is responsible for the increase in the numbers of lymphoid cells that leave the blood and enter the dermis during delayed-type hypersensitivity reactions.⁶⁶ However, Rose and Parrott,⁶⁷ have shown that enhancing vascular permeability by injection of serotonin does not promote lymphoblast migration into the skin. Furthermore, studies in our

laboratory have indicated that, although in general the amount of ear swelling (which certainly has edema as one of its major contributing factors) is correlated directly with the abilities of various radiolabeled populations to leave the blood stream to enter the inflammatory site, there are nevertheless several situations in which lymphoid cell extravasation and ear swelling show very little correlation. A striking example of this phenomenon is seen in the ability of ^{51}Cr -labeled Ox-PLN cells to enter the ears of mice undergoing either primary irritant reactions to DNFB or contact hypersensitivity reactions. Although ear swelling was comparable in the two groups of mice, the ears of the mice undergoing contact hypersensitivity reactions contained nearly 10 times more radiolabeled indicator cells than the ears of mice undergoing primary irritant reactions.

6.3.4.3. Effects of Antigen

Experiments designed to address the question of whether there is selective migration-localization and/or migration-retention of immunocompetent lymphoid cells that specifically react with antigen present in a given site can be easily misinterpreted unless some important, potentially confounding factors are taken into account. First, the administration of antigen (such as the topical application of a contact sensitizing agent to the skin of a mouse) causes massive proliferation of immunocompetent T cells in the draining lymph nodes. However, this proliferation is by no means confined to clonal expansion of T cells with specificity for the antigen in question; only a very small proportion of the proliferating cells, in fact, are responsive to antigen. Most proliferating cells are nonspecifically stimulated to divide, perhaps as a secondary response to blastogenic factors produced by the much smaller numbers of antigen-reactive cells. Carefully controlled experiments have shown conclusively that antigen-specific cells represent a very small minority of the cells infiltrating sites of cell-mediated immune reactions in the skin, such as rejecting allografts⁶⁸⁻⁷⁰ and delayed-type hypersensitivity reaction sites.^{71,72} On the contrary, the vast majority of cells present in the reaction sites are nonspecifically recruited.

Several investigators have addressed the question of whether the small numbers of antigen-specific cells that infiltrate immunologically mediated reaction sites in the skin are more likely to migrate into and/or be retained in sites where the antigen against which they are directed is present than they are to localize in sites where a different antigen is present. Sprent and Miller,⁶⁸ Tilney and Ford,⁶⁹ and Emeson⁷⁰ have all presented data that indicate that immunologically specific lymphocytes directed against antigens in skin or lymphoid cell allografts are selectively recruited and/or retained in these specific sites. Our laboratory has recently conducted carefully controlled experiments to determine whether similar significant antigen-specific localization occurs in

the dermis and/or epidermis of animals undergoing contact hypersensitivity reactions to either DNFB or Ox. Peripheral lymph node cell suspensions were prepared from mice sensitized with either DNFB or Ox, and portions of each of these suspensions were then labeled in vitro with [³H]uridine. Normal mice then received intravenous injections of a mixture of Ox-immune and DNFB-immune cells, one population of which had been radiolabeled. Groups of recipients were then challenged on the ears with either DNFB or Ox, and 24 hr later the ears were processed for autoradiographic analysis. The results of these experiments indicated a small but significant antigen-specific localization of cells in response to either antigen in the dermis of the reaction sites. When attention was focused on the radiolabeled cells present within the epidermis, we could not conclusively show antigen-specific localization for both antigens. DNFB-immune cells apparently localized specifically in the epidermis, whereas Ox-immune cells did not. Whether these observations demonstrate characteristic reactions to these specific antigens or whether they reflect the differences in the vehicles in which the challenge antigens were dissolved (acetone-olive oil for DNFB, and ethanol for Ox) remains to be determined.

6.3.4.4. Agents That Modify Cell Surface Molecules of Lymphocytes

Logically, lymphocyte movement and localization patterns must somehow involve cell membrane molecules. In fact, extensive data have been generated that indicate that alterations of cell membrane components can dramatically affect localization-migration patterns (reviewed by de Sousa⁷³). Many agents that modify cell surface membranes have been examined, including neuraminidase, trypsin, phospholipases, sodium periodate, plant lectins, sulfated polysaccharides, and metal cations (e.g., zinc and iron). Lymphocytes treated with these agents exhibit a reduced capacity to localize in the lymph nodes of recipient animals. However, the mechanisms responsible for this decreased localization are clearly variable and, in many cases, appear to be the result of sequestration of the treated cells for varying periods of time in other organs, such as the liver or spleen. Splenic sequestration alone does not appear to explain the decrease in lymph node localization of lymphocytes after they have been briefly exposed to trypsin.⁷⁴ Ford et al.⁷⁵ observed that trypsin-treated lymphocytes showed diminished localization in isolated perfused lymph nodes, suggesting that trypsin cleaves some component from the lymphocyte surface that is necessary for normal entry into the lymph nodes. When trypsin-treated lymphocytes were incubated at 17°C either in the absence of the enzyme or in cultures to which puromycin was added, they failed to recover their normal circulation properties,⁷⁶ suggesting that active synthesis of cell membrane components is required for restoration of the ability of lymphocytes to circulate

normally. There have been no published studies on the effects of agents such as trypsin on the capacity of various populations of lymphoid cells to leave the bloodstream and enter sites of inflammation in the skin.

6.3.4.5. Interaction of Lymphoid Cells with Endothelial Cells

The observation that the movement of rat lymphocytes from the blood into lymph nodes occurred virtually exclusively at the level of the postcapillary venular endothelium⁵ prompted an extensive study of these specialized endothelial cells and the interaction of lymphocytes with these cells. Other postcapillary venules are normally lined with 2 to 4 low endothelial cells and are surrounded by a delicate basement membrane. However, the histologic appearance of the postcapillary venules located in the parafollicular regions of lymph nodes, i.e., precisely the sections of the node utilized by lymphocytes in the process of circulating from blood into lymph, is strikingly different. In these regions the endothelial cells are unusually high (cuboidal) in their morphology. These high endothelial venules (HEV) are surrounded by a complex reticular sheath consisting of several layers of overlapping cytoplasmic plates derived from reticular cells. Ultrastructurally, HEV contain well-developed organelles; the Golgi area contains prominent vesicles, many mitochondria and many free ribosomes are present. In addition, these cells have enzyme and metabolic activities that are not seen in other endothelial cells, including acetyl esterase, acid phosphatase, and β -glucuronidase activity.⁷⁷ They also are distinguished by their apparently unique capacity to incorporate [³⁵S]sulfate.⁷⁸ The significance of this sulfate uptake by HEV is not completely understood. However, experiments in which partially purified sulfated material from lymph nodes was injected into normal rat skin indicated that this material caused a local inflammatory reaction, thus raising the possibility that the sulfated molecule secreted by HEV may act as a signal for lymphocyte migration.

de Sousa⁷⁹ has pointed out several problems with this hypothesis. First, it does not explain the fact that lymphocytes cross postcapillary venules in species such as sheep in which the postcapillary venular endothelium in the lymph nodes does not have a high cuboidal appearance.⁸⁰ Second, it does not explain the fact that T-cell-deficient animals, in whom the major portion of the recirculating lymphocyte pool is absent, have flat and rather poorly defined postcapillary venular endothelial cells.^{81,82} The striking feature of the endothelial cells in such T-cell-depleted animals is, however, that they change to the more typical HEV appearance after the animals are injected with either recirculating lymphocytes⁸³ or thymus grafts.⁸⁴ These observations suggest that the morphologic appearance of lymph node postcapillary venules is an effect rather than the cause of lymphocyte migration at this site. Additional observations that suggest that this may indeed be the case include the fact that T cells can

clearly leave the blood through postcapillary venular endothelium in other tissues such as the skin in which HEV are not seen. Finally, in chronic inflammatory sites in nonlymphoid tissues in which there is a persistent increase in such cell movement, such as some experimental forms of arthritis,⁸⁵ the morphology of endothelial cells has been observed to change to one resembling typical HEV.

Stamper and Woodruff and their colleagues⁸⁶⁻⁸⁸ developed and subsequently expanded an in vitro model to investigate the nature and specificity of the interaction of lymphocytes with HEV. The technique involved the layering of suspensions of lymphoid cells over sections of lymph nodes fixed with glutaraldehyde. They observed that, under appropriate conditions, lymph node, splenic, and thoracic duct lymphocytes, but not thymocytes or bone marrow cells, adhered to the HEV of the fixed sections. The adherence required living, metabolically active lymphocytes and was calcium dependent. The binding was inhibited by pretreatment of the lymphocytes with cytochalasin B, suggesting that the binding depended on contractile mechanisms generated by cytoplasmic microfilaments.

Kuttner and Woodruff⁸⁹ have recently shown that thoracic duct T cells and B cells adhere equally well to HEV in vitro; however, recent studies by Butcher et al.⁹⁰ have presented compelling data that different lymphoid populations have different abilities to bind selectively to the HEV present in different lymphoid tissues. In these experiments, murine lymphocyte suspensions from either Peyer's patches or PLN were found to bind preferentially to HEV in sections of Peyer's patches or PLN, respectively. That is, about 1.4 times more Peyer's patch lymphocytes than PLN lymphocytes bound HEV in Peyer's patches and, conversely, twice as many PLN lymphocytes bound to HEV in PLN. These results clearly demonstrated some organ specificity in the interaction of whole lymphocyte populations with HEV; nevertheless, they did not define the degree of specificity possible at the level of individual cells. In an attempt to define this degree these investigators examined the binding properties in vitro of relatively homogeneous lymphocyte populations, i.e., T-cell lymphomas from AKR or C57BL/6 mice. Four lymphomas exhibited selective affinity for Peyer's patch HEV, binding to these structures approximately 3 times as well as they bound to the HEV in PLN sections. Conversely, two lymphomas adhered approximately 3 times as well to HEV in PLN as they did to the HEV in Peyer's patches. This selectivity of binding to HEV by T-cell lymphomas suggests that appropriately programmed lymphocytes may be capable of nearly absolute discrimination between Peyer's patch and PLN HEV, presumably by virtue of specific receptors for distinctive determinants expressed by HEV in different organ sites. These observations of organ-selective adherence to HEV by different populations of lymphoid cells correlate extremely well with the compelling in vivo data that indicate that organ-specific migration-localization of radiolabeled lymphoid cell populations can be demonstrated.

6.3.4.6. Influence of Ia-Bearing Cells

Janossy and co-workers,⁹¹⁻⁹³ utilizing monoclonal antibodies to T cell subset antigens as well as antibodies directed against Ia antigens and employing the technique of immunofluorescence of frozen sections, studied the staining patterns with these reagents in various lymphohematopoietic organs, the gut, and rheumatoid synovial tissue. OKT-4⁺ (helper-inducer) T cells predominated in the thymic medulla, the parafollicular regions of lymph nodes and tonsils, the jejunal lamina propria, and rheumatoid synovial tissue. Alternatively, OKT-8⁺ (suppressor-cytotoxic) T cells were found in the highest relative proportions (compared to OKT-4⁺ cells) in the bone marrow and in jejunal epithelium. The most striking observation in these studies, however, was the finding of an intimate microanatomical relationship between OKT-4⁺ T cells and dendritic and/or macrophage-monocyte-like cells that expressed large amounts of Ia-like antigen on their surface. Such Ia⁺ cells were virtually absent from areas such as jejunal epithelium that contained large numbers of OKT-8⁺ T cells. In the parafollicular regions of the tonsils and lymph nodes in which approximately 70% of the T cells were OKT-4⁺ and 30% were OKT-8⁺, it appeared that the Ia⁺ interdigitating cells were preferentially surrounded by OKT-4⁺ rather than by OKT-8⁺ T cells. As previously mentioned, it is already known that at least a proportion of interdigitating cells contain Birbeck granules and are related to, if not identical to, epidermal Langerhans cells and circulating “veiled” cells in afferent lymph.⁵⁰ All of these cells express large amounts of Ia-like antigens.⁹⁴

Several investigators have documented both by electron microscopy and by immunofluorescence of biopsies from patients with the epidermotropic variants of CTCL that there is intimate association of atypical T cells with Ia⁺ Langerhans cells within the epidermis and dermis.⁹⁵⁻⁹⁷ Finally, the recent work of Haynes and co-workers (personal communication) of the cutaneous infiltrates in patients with allergic contact dermatitis has also indicated a close association within the epidermis of T cells, most of which were OKT-4⁺ cells, with Ia⁺ dendritic cells.

6.3.4.7. Chemotactic Factors

The phases *migrating into* or *signaling* imply that positive vectorial movement is involved in lymphocyte locomotion and therefore further imply that lymphocytes can detect and move along a concentration gradient. Therefore, two very relevant questions are whether chemoattractants affect the locomotion of lymphocytes and, if so, whether that effect is chemotactic or chemokinetic. *Chemotaxis* has been defined as a reaction in which the direction of locomotion of cells or organisms is determined by substances in their environment;⁹⁸ *chemokinesis* is a reaction in which the speed or frequency of locomotion of cells or organisms is determined by substances in their environment.⁹⁹ The major difference between these phenomena is one of oriented or vector-related motion

versus the speed or frequency of motion. As pointed out in a recent comprehensive review,¹⁰⁰ chemokinetic reactions in both small lymphocytes and lymphoblasts have been documented by many investigators; however, unequivocal evidence for the ability of these cells to show chemotaxis has been difficult to obtain. Part of the difficulty appears to be technologic: The techniques designed for studying chemotaxis of polymorphonuclear leukocytes and monocytes *in vitro* may need to be modified for the study of lymphocytes and lymphoblasts. At the present time, Parrott¹⁰⁰ believes there is insufficient *in vitro* data to draw meaningful conclusions that can be applied directly to the interpretation of *in vivo* movement of lymphocytes. However, available data suggest that chemokinetic effects of chemoattractants (including antigens) may be of more fundamental importance when attempting to explain lymphocyte migration patterns than specific chemotactic effects. It is possible, for example, that soluble chemokinetic factors are important for increasing the overall locomotion of lymphocytes, and that any selective retention of those lymphocytes in a particular microenvironment may be attributable to the presence of some other local factor (such as the recognition of Ia molecules or of molecules like macrophage inhibitory factor).

6.3.4.8. Experimental Tumor Metastasis: A Model System for Examining Factors That Influence Organ-Specific Migration

Over the past several years, a fascinating series of studies has been conducted utilizing a mouse melanoma designated B16. These studies have been directed at elucidating the mechanisms involved in the capacity of malignant cells to metastasize to different organs. We will review briefly some of the major findings in this model system because it is likely that other cells, including both normal and malignant lymphocytes, may use similar strategies in directing their patterns of migration.

The B16 melanoma grows progressively in the skin at the site of inoculation into normal syngeneic (C57BL/6) mice, and metastasizes widely to multiple organs, such as the lung, brain, liver, spleen, and ovary. Fidler, Nicolson, and co-workers initiated an ingenious series of studies in which numerous different organ-specific sublines of the original tumor were established.¹⁰¹⁻¹⁰⁵ This was achieved by passaging serially the metastatic nodules that appeared in different organs into separate groups of mice. Thus, the lung metastases were passaged in one group of animals, the brain metastases in another group, and the ovarian metastases in a third group. When these new recipients developed tumors, the lung tumors in recipients of the original lung metastases were again passaged in new animals, the brain metastases occurring in the animals receiving the original brain metastases were passaged in other animals, and so forth. After repeated serial passage in this manner, a striking organ-specific metastatic profile was established in which the lung-specific subline would give rise only to lung metas-

tases in recipient mice, the brain-specific subline metastasizes only to brain metastases, and the ovary-specific subline metastasizes only to ovarian metastases. Two explanations for the organ-specific behavior of the variant lines were considered. Either the original tumor line was heterogeneous and comprised of several different metastatic variants or the selected sublines represented tumor cells that had undergone organ adaptation during the selection process. Fidler and Kripke¹⁰⁶ examined this question by cloning the original unselected tumor line *in vitro*. When the individual clones were examined for their capacity to form experimental pulmonary metastases, it was found that each clone differed dramatically in its ability to do so; some clones formed multiple lung metastases and others formed no lung metastases whatsoever. These experiments demonstrated that the potential to form lung metastases was inherent in certain clones within the unselected tumor cell population.

Nicolson and co-workers^{104,105} examined some of the organ-specific B16 melanoma sublines for differences in cell surface proteins accessible to lactoperoxidase-catalyzed iodination. Autoradiographs made from polyacrylamide gels showed that the profile of cell surface proteins of the brain-specific subline was identical to that of the unselected line, except for two proteins of approximately 95,000 and 100,000 daltons that were clearly more intensely stained on the gels of the brain-specific subline. In contrast, the staining of the 95,000- and 100,000-dalton proteins on gels of the ovary-specific subline was similar to the original unselected subline, but proteins with molecular weights of approximately 140,000 and 150,000 daltons were clearly darker in gels of the ovary-specific subline.

The capacity of melanoma sublines with a very high metastatic potential to adhere to other cell types including endothelial cells has been shown to be significantly greater than that of variant sublines selected for their inability to metastasize.¹⁰⁷ Additional studies suggest that lung-specific melanoma cells apparently have the capacity to recognize selectively determinants existing on parenchymal and endothelial cells.¹⁰⁵ In these experiments, a suspension of normal syngeneic lung cells was mixed on a gyratory shaker with either the unselected or lung-specific tumor sublines, and cell aggregation was assessed within 5 min. Within this very short period of time, the highly metastatic lung cell subline aggregated with the lung tissue into a single large clump, whereas the unselected cells were only slightly aggregated. In contrast, organ cells from normal nontarget tissues, such as kidney or spleen, were not aggregated above the levels of the controls by either of the sublines. These results suggest that patterns of organ-specific tumor colonization occur because of specific adhesive interactions between cells; furthermore, virtually all of the suspended lung cells and lung-specific melanoma cells were aggregated into a single clump, suggesting that recognition determinants exist on parenchymal cells as well as on endothelial cells.

6.4. Integration of Peripheral Lymph Nodes with T Lymphocytes and the Skin

The existence of a distinct lymphocyte migration pathway through the gut and its associated organized lymphoid tissue (such as Peyer's patches and mesenteric lymph nodes) has been generally acknowledged since 1969 when Griselli et al.¹⁰⁸ showed that radiolabeled lymphoblasts obtained from mesenteric lymph node (MLN) of rats would, following adoptive transfer to normal recipients, accumulate mainly in the gut wall and MLN. In contrast, lymphoblasts obtained from PLN showed selective localization in PLN instead of the gut and MLN. The concept that subsets of lymphoblasts have different patterns of migration has been repeatedly confirmed in other species including mice^{109–112} and sheep.¹¹³

It was initially thought that these migration patterns were attributable to distinct differences in migration pathways of B lymphoblasts within these tissues. Several laboratories demonstrated that IgA B lymphoblasts from either MLN or PLN would localize selectively in the gut of recipient animals, whereas IgG B lymphoblasts from either of these two sources would localize preferentially in PLN.^{6,112,114} However, it is clear that T lymphoblasts from these tissues also show striking organ-specific localization. Guy-Grand et al.⁶ first demonstrated this in mice using T lymphoblasts isolated from the thoracic duct. Rose et al.^{109,110} produced more definitive data using T lymphoblasts isolated from either MLN or PLN. These investigators showed that MLN T lymphoblasts migrated preferentially to MLN and normal gut mucosa, whereas T lymphoblasts from PLN showed a significant preference for PLN rather than normal gut or MLN.

As previously discussed, T lymphoblasts from PLN are more apt to enter sites of cutaneous inflammation than PLN small T cells. In contrast, T lymphoblasts from mesenteric lymph nodes¹⁰⁹ are less apt to localize in inflamed skin than T lymphoblasts from PLN. These same investigators¹¹⁰ demonstrated that although T lymphoblasts from PLN can enter sites of inflammation in tissues other than the skin, such as the inflamed gut of mice infected with *Trichinella spiralis*, they clearly display a lesser tendency to localize there than T lymphoblasts from MLN. Most importantly, when confronted with inflammatory sites in both the skin and the gut in the same animal, the T lymphoblasts from MLN selectively migrate into the inflamed skin rather than into the inflamed gut.¹¹⁰ This is the single most important piece of evidence to suggest that T lymphocytes generated in peripheral lymph nodes are drawn to skin and not gut through a positive affinity for some determinant expressed therein.

Quite apart from considerations of the migratory patterns of T lymphocytes, work with chronic, high-dose UV irradiation of mice raised the intriguing possibility that a migratory pathway may also exist for Langerhans cells. This

idea stems chiefly from the work of Kripke, Noonan, Greene, and their various collaborators.^{115,116} The arguments go as follows: Chronic, high-dose irradiation of mice with UVB radiation produces an altered immunologic status in which attempts to induce hapten-specific delayed-type hypersensitivity to hapten-conjugated syngeneic lymphoid cells fail. Spleen cells harvested from these chronically irradiated animals contain nonspecific suppressor cells whose function is revealed when they are transferred into unirradiated, naïve recipients. Moreover, attempts to immunize these animals with haptens produces a state of specific unresponsiveness that is also transferrable and mediated by suppressor T lymphocytes. The hypothesis has been generated that the basis of UV-radiation-induced impairment of systemic immune responsiveness to haptens involves perturbation of antigen-presenting cells within the skin. It is implied, therefore, that these putative antigen-presenting cells must, under physiologic conditions, migrate to the spleen. Chronic UV irradiation reveals these cells through its perturbation of their normal functional activity. Langerhans cells may represent the migrating cell type and, therefore, may recirculate in the manner of lymphocytes from skin to systemic sites. Since it is likely that Langerhans cells are synonymous with or represent a subpopulation of dendritic cells, the observations of Anderson et al.,¹¹⁷ that a small but significant population of dendritic cells is a regular passenger in the flow of lymph through the thoracic duct are significant.

6.5. Consideration of the Mechanisms Whereby Cells Become Skin-Specific

6.5.1. Relative Importance of Microenvironment in Which Proliferation Takes Place

The microenvironment in which a particular normal T cell is stimulated into lymphoblast transformation or division seems to be more important in ultimately determining the preference of the cell for a similar microenvironment than the specific nature of the antigen(s) that causes (either directly or indirectly) the cell to divide. Studies in mice¹¹⁸ indicate that stimulation by some antigen may be necessary for normal T cells to express the capacity to localize selectively in a particular site. Small unstimulated T cells from PLN and MLN exhibit no organ-specific migration preference, in contrast to the selective localization exhibited by T lymphoblasts from these same tissues. Furthermore, studies of the migration pattern of T lymphocytes from adult and fetal sheep^{119,120} suggest that antigenic stimulation and subsequent lymphoblast transformation or division may be a critical event in expanding the population

of T cells with organ-specific migration patterns. Alternatively, the meticulous work of Guy-Grand et al.¹²¹ strongly suggests that the migratory behavior of T lymphoblasts is independent of the specific antigenic stimulus, but is heavily dependent on the microenvironment in which that stimulus for proliferation-differentiation took place. In these experiments, thymocytes from normal strain A donors were injected intravenously into lethally irradiated (AXB)F₁ recipients. Some of the thymocytes from the parent strain localized in PLN where they confronted strain B histocompatibility antigens. Other thymocytes localized in MLN, where presumably identical strain B histocompatibility antigens were present. In both sets of lymph nodes, the response to alloantigen included the proliferation of T cells of strain A: Five days later, when this T cell proliferation was at its peak, single cell suspensions were prepared from either the PLN or the MLN. The suspensions were then incubated in vitro with [¹²⁵I] iododeoxyuridine to radiolabel selectively the proliferating cell populations. Each of these two radiolabeled suspensions was then injected intravenously into groups of normal strain A recipients, and the localization of the radiolabeled cells in either the PLN or MLN was determined 24 hr later. The results showed conclusively that the T lymphoblasts proliferating in PLN in response to strain B histocompatibility antigens showed a distinct preference for localizing in PLN as opposed to MLN, whereas T lymphoblasts responding to the same strain B histocompatibility antigens within the MLN showed a preference for localizing in MLN as opposed to PLN. Thus, the existence of unique recirculatory pathways, such as GALT and SALT, does not depend exclusively on the unique aspects of the antigens in these sites that cause proliferation and clonal expansion of the cells (although the types of antigens confronted in these locations under normal circumstances may be very different); an expanded population of cells with an increased affinity for a similar microenvironment may develop as a result of the proliferation and clonal expansion in a particular microenvironment.

How this phenotypic alteration seen within populations of heterogeneous lymphocytes is expressed at the level of a single cell is at present unknown. There are at least two major possibilities. The first can be conveniently termed *the imprinting model*. It is possible that a given cell with specificity for a given antigen has no particular microenvironmental tropism and that the ultimate tropism displayed by its progeny is dictated by the particular microenvironment in which the antigen happened to be confronted. In this case, given more than one possible site for meeting antigen, i.e., the cell could meet with a particular antigen in either microenvironment X or Y, chance alone would determine the location. If the cell proliferated in microenvironment X, its progeny would display increased tropism for microenvironment X; if it proliferated in microenvironment Y, its progeny would have an increased tropism for microenvironment Y. The second major possibility can be termed *the selective migration-*

clonal expansion model. In this model, a population of cells with specificity toward the same antigenic determinant has subpopulations, each of which already has a tropism for a particular microenvironment. In this case, the initial localization of a cell in one particular microenvironment where it perceives antigen and undergoes clonal expansion, as opposed to another microenvironment where it would perceive the same antigen, is not a random event. The apparently random and equal initial localization into either microenvironment X or microenvironment Y by a heterogeneous population of cells would, in reality, be a selective localization of some cells in microenvironment X and the equally selective localization of an equivalent number of other different cells in microenvironment Y. After clonal expansion of these subsets in their preferred sites, the tissue tropism of cells obtained from microenvironment X would be greater for microenvironment X than for microenvironment Y; and vice versa. The previously described experiments, in which cloned progeny from an unselected population of melanoma cells exhibited strikingly different capacities to metastasize to the lung, suggest that, at least within such populations of malignant cells, this heterogeneity already exists. Similar experiments with normal lymphoid cells have not yet been attempted.

The demonstration that T cells that have undergone lymphoblast transformation, in particular lymphoid organs (e.g., PLN versus MLN), display an increased tropism not only for that particular lymphoid organ but also for the major tissue directly connected (by afferent lymphatics) to those lymph nodes (i.e., skin in the case of PLN and gut in the case of MLN) suggests a distinctive signal and/or membrane-associated determinant within each of these two major spheres of influence that directs the migration of discrete subpopulations of T cells. The possibility that Ia antigens expressed on bone-marrow-derived monocyte–macrophage cells could act as such an integrating molecule has already been mentioned in regard to the selective attraction of functionally and phenotypically discrete subpopulations of T cells (see Section 6.3.4.6, *Influence of Ia-Bearing Cells*). If one were to suggest that Ia-like determinants are also responsible for the capacity of peripheral T lymphoblasts to localize selectively in PLN and skin as opposed to MLN and gut, one would have to postulate that there are discrete or distinctive Ia determinants expressed by cells within each of these major spheres of influence. Evidence for such regional differences in Ia expression by macrophages already exists.¹²²

We have also considered the evidence suggesting that distinctive determinants expressed by endothelial cells in different lymphoid tissues may direct the adherence and subsequent migration-localization of subpopulations of T cells. The biochemical nature of these determinants on HEV is, at present, poorly understood. It is possible that endothelial cells, in the skin for example, express some Ia-like determinants similar to those expressed in PLN.

6.5.2. Potential Contributions of Factors Released by and/or Expressed on the Surface of Keratinocytes That Influence the Migration-Localization of T Cells

Ia-like molecules have been detected on the membrane and within the cytoplasm of keratinocytes in various conditions. These include inflammatory dermatoses such as lichen planus,¹²³ and CTCL¹²⁴ in man, and in graft-versus-host diseases in both rats and man.^{125,126} It is still not clear whether these molecules are indeed synthesized by the keratinocytes themselves, or whether they are passively acquired, perhaps being produced by Ia⁺ Langerhans cells, by other Ia⁺ bone-marrow-derived monocyte-macrophages, or by activated T cells.²⁷⁻¹²⁹ Even if definitive experiments clearly show that the Ia-like molecules are passively acquired by the keratinocytes, the possibility still exists that these molecules could act as signals or determinants for the localization of T cells able to recognize them.

Even more intriguing, however, are the observations that suggest that products of keratinocytes may alter the state of differentiation of T lymphocytes, as well as the cell surface antigens they express, and conceivably, therefore, their migration patterns. Rubenfeld and co-workers¹³⁰ recently investigated the effects of cocultivating normal human E-rosette⁺ peripheral blood T cells with a variety of allogeneic cells, including cultured normal human keratinocytes, a fibroblast cell line, a mammary epithelium line, and normal peripheral blood mononuclear cells. These investigators examined the T cells after cocultivation for the presence of terminal transferase. This enzyme, which is found in lymphoid cells, appears to be the earliest identifiable marker of cells that can undergo maturation directed by the thymus.¹³¹⁻¹³³ It is most readily demonstrable only in thymus and in bone marrow and is not found in mature normal peripheral T cells in the blood, lymph, or spleen.^{134,135} The results of these studies indicated that keratinocyte cultures alone were capable of inducing the expression of terminal transferase in presumably mature, differentiated peripheral blood T cells. Rubenfeld has suggested the possibility that the induction of this DNA-altering enzyme in peripheral T cells may be a sign that epidermis is a "peripheral tissue with a capacity to expand the diversification of the T cell pool."¹³⁰

Haynes and co-workers¹³⁶ suggest that normal peripheral blood T cells coming under the influence of factors within the skin (perhaps produced by keratinocytes) may alter their cell surface antigens. Using an indirect immunofluorescence technique with monoclonal antibodies directed against various antigens expressed on subpopulations of T cells, these investigators examined the cell surface phenotypes of lymphocytes found within the epidermis in skin biopsies of patients with various benign inflammatory dermatoses, including

allergic contact dermatitis. They found that many of the intraepidermal T cells expressed the Thy-1 antigen. Normally, the expression of this antigen on lymphocytes is essentially confined to immature, subcapsular thymocytes; it is not readily detected on normal peripheral blood T cells or lymph node cell suspensions.¹³⁷ One interpretation of this finding with relation to allergic contact dermatitis reaction sites is that mature T cells, under the influence of the epidermis, may have undergone a type of “dedifferentiation process,” resulting in their expression of a cell surface antigen characteristically expressed by a less differentiated cell. The possibility that the induction of terminal transferase activity within the cell is associated with the expression of (or re-expression of) cell membrane determinants is intriguing. Perhaps keratinocytes can induce the expression of cell surface molecules on T cells within their vicinity thus programming these cells (or possibly reprogramming them) to exhibit subsequently an enhanced tropism for the skin, in general, and the epidermis, in particular.

Alternatively, it is possible that the expression of Thy-1 antigen on cutaneous T cells reflects the selective arrival of cells that have acquired the Thy-1 antigen in some other site, such as the PLN. McKenzie and Fabre¹³⁷ and Ritter and Morris¹³⁸ have noted that lymph node postcapillary venules (the site of emigration of lymphocytes from blood into lymph node) express the Thy-1 antigen. The Thy-1 antigen may somehow be involved in the lymphocyte–endothelial cell interaction necessary for lymphocyte recirculation and some T cells, as a result of interacting with Thy-1⁺ endothelial cells, may acquire this antigen on their surface. These cells may undergo proliferation in the lymph node and then move into the blood stream from which they subsequently can enter cutaneous inflammatory sites. The major difficulty with such an hypothesis, however, is that Thy-1⁺ cells are not readily detectable in peripheral blood.¹³⁶ In any event, the expression of the Thy-1 antigen on (1) T cells within the skin, (2) cutaneous endothelial cells in inflammatory sites,¹³⁶ and (3) postcapillary venular endothelial cells in peripheral lymph nodes supports the hypothesis that a special relationship exists between T cells, the skin, and peripheral lymph nodes.

6.6. A Comparison of SALT and GALT

Both SALT and GALT appear to attract unique and nonoverlapping subpopulations of T lymphocytes and B lymphocytes. The attractive forces seem to be independent of antigen-recognizing potential or the process of antigen recognition, indicating that a different set of ligand–receptor interactions is responsible. In neither instance has the molecular nature of lymphocyte–tissue interaction been elucidated, nor is anything known specifically about the precise site within each tissue that this interaction takes place. One might reason a priori

that endothelial cells of capillaries and postcapillary venules could perform this role, however, there is no direct evidence that this is the case. A second explanation would be that lymphocyte migration through endothelial surfaces into extravascular spaces is random in both skin and gut, but that selective affinity is engendered through specific interactions between lymphocytes and parenchymal (epithelial) cells of each tissue. A third explanation (in reality a derivative of the second) is that a unique cell type, perhaps the Langerhans cell in epidermis, provides a force that constellates lymphocytes uniquely within skin.

Both GALT and the proposed SALT possess an integrating and relatively unique set of draining lymph nodes. For the former, mesenteric lymph nodes have been clearly identified; for the latter, diffusely distributed subcutaneous lymph nodes, including axillary and inguinal nodes, are thought to subserve this function. Evidence suggests that the affinity described for subpopulations of lymphocytes for each tissue is also a property of the draining lymph nodes, since these structures are greatly enriched for lymphocytes having special affinity for the tissues they drain. Presumably, the intermediaries responsible for unique localization of lymphocytes are the high endothelial cells of the postcapillary venules of the peripheral and mesenteric nodes, respectively.

GALT and SALT possess within their epithelial components antigen-presenting capabilities. In the case of SALT, epidermal Langerhans cells, both *in vitro* and *in vivo*, present antigens applied to the cutaneous surface, to recirculating lymphocytes. Whether this presentation takes place within the epidermis, or whether these cells drop down through the dermal–epidermal junction and carry their immunogenic signal to the draining lymph node is unclear; both scenarios may, in fact, be true. In the case of GALT, a specialized epithelial cell, the M cell, appears to carry out a role in antigen presentation.¹³⁹ Considerably less is known about this cell; besides being of epithelial origin, it seems to permit and/or promote the passage of antigenic material from the gut lumen through the epithelial surface to the Peyer's patches directly beneath. Little is known about cell surface determinants on M cells; attempts to purify or enrich cellular suspensions for these cells and then test *in vitro* for antigen-presenting potential have not been successful.

Although GALT and SALT share epithelial capabilities for antigen presentation, this property is distributed differently in the two tissues. In the case of skin, antigen-presenting Langerhans cells appear to form a pervasive anastomosing web of dendritic processes that in the aggregate confer upon the entire cutaneous surface, in a relatively homogeneous fashion, the capacity to accept, process, and present epicutaneously delivered antigens. Only the cornea seems devoid of Langerhans cells. By contrast, specialized portions of the gut epithelial surface that function in antigen-presentation are discontinuously deployed. Unique associations between lymphoid tissues and gut epithelium are found in the tonsillar tissues of the oropharynx, in Peyer's patches, and in the appendix. M cells, which are epithelial cells specialized for antigen presentation, seem to be

found only at these sites. Thus, although the entire integument seems equipped for the function of antigen recognition, only a minor portion of the alimentary surface is similarly endowed. These differences may relate in some direct fashion to obvious differences in the functional properties of skin and gut. The skin serves exclusively as a barrier, whereas the gut serves predominantly, if selectively, to permit and even promote the entry of exogenous materials into the body. Reasoning teleologically, it would seem consistent, therefore, for the skin to provide a continuous barrier to the intrusion of antigenic materials at any site; by contrast, the gut, for whom creation of such a monolithic barrier would compromise its functional intent, is outfitted with specialized local areas for recognition and processing of antigenic materials. Each epithelial surface is confronted with a unique set of problems relating to its antigen-presenting function. The skin is assaulted with sunlight containing UV radiation, with significant changes in ambient temperature, and with a wide range of chemical substances to which it must raise a molecular barrier. Antigen-presenting Langerhans cells appear to insinuate themselves at the furthest extremity of the epidermal intercellular space, just beneath the stratum corneum where they can capture antigenic materials immediately upon penetration of this molecular barrier. The skin's capacity to melanize protects the Langerhans cells from the injurious effects of UV irradiation.

Finally, it is possible that SALT and GALT each possess the capacity to enlist lymphocytes whose antigen-recognizing repertoires are unique and only partially overlapping, i.e., certain antigenic specificities will be represented by specific antigen-recognizing lymphocytes with affinity for skin, but not gut, and vice versa. In addition, certain other antigenic specificities may be represented by two sets of lymphocytes with similar antigen-recognizing repertoires, one dedicated to SALT, and a cohort population dedicated to GALT. Evidence bearing on this point is incomplete.

6.7. The Nature and Biologic Meaning of SALT

Skin-associated lymphoid tissues comprise a specialized set of (1) antigen-presenting cells within the epidermis, Langerhans cells, and (2) subpopulations of recirculating T and non-T lymphocytes whose interactions with each other and whose recognition of antigen are determined largely by their anatomic association with keratinocytes in the epidermis. Keratinocytes, by their location and perhaps by virtue of the pharmacologic properties of their locally secreted molecular products, impose on this multicellular association functional properties that provide skin with its own regional sphere of immunologic influence. Integration of this multicellular system is achieved through the draining regional lymph nodes. As a consequence, lymphoid cells dedicated in this manner and in this microenvironment have a proclivity for recirculating selectively to the skin,

and its associated draining lymph nodes, a migratory pattern determined presumably by unique properties of the vascular endothelial cells of the dermis and the parafollicular nodal cortex. By implication, keratinocytes, whose functional properties endow skin with its unique physiologic characteristics, orchestrate the various lymphoid cells and tissues into a unified immune system that serves the special needs of the skin. In this manner, the cutaneous surface is endowed with immunologic responsiveness that is uniquely suited to its special needs.

Because of its anatomic location as the physical and functional barrier between the organism and the environment, skin is likely to be confronted by a unique spectrum of antigenic demands; the range of possible antigens that come in contact with the cutaneous surface is undoubtedly different, at least in part, from the range of antigens that confront the gut, or the respiratory tract. Consequently, SALT probably is comprised of a unique and complementary set of immunocompetent lymphocytes whose spectrum of antigen-recognizing capabilities matches that of its antigenic experience. Whether this unique set of immunocompetent lymphocytes is committed ontogenetically to a SALT pattern of existence, or whether the pattern is a consequence of antigenic experiences within the cutaneous microenvironment is unclear at present.

Skin must also retain its capacity to meet immunologic challenge while weathering the deleterious effects of UV irradiation and other forms of physical trauma. A pervasive, anastomosing network of dendritic, antigen-presenting Langerhans cells at the very extremity of the extracellular space that exists immediately beneath the stratum corneum ensures the effectiveness of antigen presentation. The epidermal process of melanization protects the UV-radiation-sensitive Langerhans cells. Immunogenetic factors also govern the susceptibility of Langerhans cells to the deleterious effects of sunlight.

When SALT fails, the organism is bereft of a specialized immune surveillance mechanism that, under physiologic circumstances, monitors the epidermis constantly for evidence of malignant transformation, whether of keratinocytes, melanocytes, or of Langerhans cells, themselves. In the wake of a disassembled SALT, actinic keratoses, squamous cell and basal cell carcinomas, malignant melanomas, and perhaps even histiocytosis X would escape destruction by the surveillance system. In addition, cutaneous viral and fungal infections, which also would be considered within the purview of SALT, would not be dealt with decisively, thus exposing the organism to the serious threat of persistent infection.

Malignant transformation of the lymphoreticular cell components of SALT can also occur. Infiltration of these transformed cells and attendant inflammatory reactions produce the harmful effects observed in cutaneous lymphoma syndromes.

Allergic contact dermatitis, the bane of outdoorsmen and gardeners, is an important, if nonlethal, expression of the effectiveness of SALT. The agents

capable of inducing and eliciting allergic contact dermatitis seem innocuous in their own right, and therefore the inflammatory reaction they produce seems unimportant. However, it is a testimony to the pervasive scrutiny of SALT that uroshiol applied in trivial amounts to the cutaneous surface of a person sensitive to poison ivy is readily detected, thus raising the hope that similarly effective detection systems monitor for tumor-associated neoantigens and for antigenic determinants expressed on surfaces of virus-infected cells. In reality, allergic contact dermatitis seems a modest price to pay for the protection SALT affords.

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Factors That Influence Photocarcinogenesis

P. D. Forbes and R. E. Davies

7.1. Introduction

Laboratory animals have been shown to be susceptible to carcinogenesis by UV radiation. For example, the inbred strain of hairless (but immunologically competent) HRA/Skh mice normally develop very few spontaneous tumors; among mice that live for 2 yr, only about 6% have skin tumors, in a distribution of about 4 papillomas to 1 sarcoma to 1 carcinoma. However, when these animals are exposed to UV radiation under certain circumstances, all exposed animals can develop multiple, aggressive tumors in less than 1 yr. The proportion of affected mice, the multiplicity of tumors, the tumor types, and growth rates are all strongly influenced by circumstances of exposure and related events. This chapter considers some of those circumstances.

7.2. Radiation

The amount and type of radiation are conceptually distinct terms, but in nature they do not vary independently; under laboratory conditions amount and type can be treated as experimental variables.

7.2.1. Amount

Dose traditionally is used to mean the amount of radiation integrated over time. Dose is thus the product of radiation intensity multiplied by duration of exposure and, for a given dose, the intensity and duration may be varied as reciprocals of each other. As used in this chapter, *dose* refers to the accumulated quantity of radiation reaching a place normal to the surface of the skin. (Unre-

strained mice represent a special case in which various skin surfaces are exposed or shadowed by the animal's body.) The actual amount of radiation reaching and penetrating biologic materials at defined depths is not practically measurable at this time. In chemical, physical, and microbial systems, where the absorber (target) bears a specified geometric relationship to the radiant beam, irradiance integrated over time is expressed by the term *fluence*.¹ (See Chapter 3 for further definition of this term.)

Photocarcinogenesis involves both a stimulus and a response. The stimulus is UV radiation, sometimes accompanied by other radiations including light, and sometimes accompanied by test chemicals. Both because of the useful analogy involved and because chemical agents are to be discussed later, we will introduce this section with a description of stimulus and response as they apply to both chemicals and radiation.

In any experiment designed to measure the relationship between stimulus and response, the definition of both is critical. In some cases, such definitions appear obvious: for example, what proportion of animals die (response) when compound A is administered at various levels (dose)? Even here, the definitions need to be further clarified. Are the effects acute (death in one or a few days), subacute (death within weeks resulting from, for example, immune suppression or nephrotoxicity), or life-shortening and chronic? Obviously our response parameter requires a time-integral component. The definition of dose must include a route qualification, e.g., oral, subcutaneous, intravenous, and a mode of delivery. (Is it delivered as a single bolus, such as gavage or intravenous injection, or is it protracted in feed, or as an intravenous drip?) Here again, the element of a time is introduced. Thus, dose is the product of intensity (dose/unit time) and duration. Intensity may need further qualification (e.g., concentration times volume) and the variable of duration can become complicated if it approaches the value defined in the response term.

In chronic experiments, these complexities are compounded. In typical long-term carcinogenesis studies, time is implicit in the response parameter, whether it is measured as cumulative lifetime events, as prevalence at an arbitrary end point, or as average time to response. Such studies often involve chronic treatment with the test material, and the dose that produces an observed effect is obviously related to the time over which it was administered. When the number of deaths and responses is small at the (arbitrary) time of response measurement, the integral dose (amount per application \times number of applications) can be useful. However, when most animals respond, or if the measure of response is a time variable (average time to tumor development), the dose integral is partially defined by the response and acquires undesirable statistical properties. The usual solution to this problem is to express the dose axis in terms of delivery rate, e.g., concentration in feed, mg/day. This, however, is a measure of intensity rather than dose, and the resulting relationship is that of response to intensity with the proviso that treatment is chronic.

Corresponding problems arise in the study of photocarcinogenesis. However, there is much less ambiguity concerning the duration of a periodic treatment. The radiation source is either on or off, and there are no questions relating to blood levels, compartmentalization, food consumption or other such factors. This increases experimental flexibility, but also increases and emphasizes the time component of dose. A given quantity of radiation can be delivered in minutes or months, continuously or at intervals, and the repetition rate can also be varied.

In many photocarcinogenesis experiments, most surviving animals acquire tumors at some point. When this is the case, the most useful observable treatment effect may be a change in the time required to produce tumors. This is particularly true if the range of doses is so great that no single arbitrary end point (truncation) provides separable evaluations for all groups. For example, lower doses may fail to produce any tumors until after all members of higher-dose groups are affected; prevalence² measured at any single time may be, for example, 0%, 0%, 100%, 100% for ascending dose groups. Much more information is obtained from such studies by measuring the time required to produce a definable response, even though this leads to the dose-definition problem mentioned previously.

There are several ways to define response in a carcinogenesis experiment: proportion of affected animals, average number of tumors per animal, or average tumor volume per animal. (Each of these requires explicit definition of tumor as a countable or measurable response, usually based on criteria of morphology, size, and stability.) Unless the study is directed specifically toward some aspect of tumor growth or individual susceptibility, the animal should be considered as the experimental unit, and all animals should be weighted equally by counting each as affected or unaffected. The proportion of affected animals, plotted as a cumulative distribution function against time, provides one description of the results that can be compared to another group by, for example the Kolmogorov-Smirnov³ two-sample test. An alternate approach that is particularly useful when there is appreciable mortality is to combine a series of randomization tests created from time-slices of the experiment, using the methods of Peto et al.⁴ for mortality-independent tumors.

Techniques such as these are useful for assigning a level of confidence to the differences between groups. In dose-response studies, however, what is needed is a measure of the magnitude of response; the statistical reliability of the measure is also important. A common approach to this problem is to assume that the cumulative distribution function approximates a particular shape (often normal or log-normal). The data are fitted to the prescribed shape by a least-squares technique, and the location and dispersion parameters of the fitted function provide magnitude and reliability estimates, respectively, for the response. These estimates are somewhat troublesome when there is considerable intercurrent mortality or when the data are right censored, i.e., a number of animals fail to

acquire tumors while surviving for the entire experiment. Most analytical models of this type are also quite sensitive to the effects of outliers, animals that for no discernible reason acquire tumors much earlier or much later than the rest of the group; the basis for excluding such animals from the calculations is usually arbitrary, but the alternative is often a poor fit of the model to the data. Alternative methods of describing the group response have been designed to avoid most of the assumptions involved in explicit curve fitting. That issue is beyond the scope of this chapter, however, and will not be considered further here.

As we indicated previously, there is little ambiguity concerning the duration of a single treatment with radiation, but there is a very important time component in the delivery of dose. Consequently, we use the following terms to emphasize the distinction associated with the delivery of a dose of radiation: (1) An *irradiation cycle* is defined as the smallest unit of time that encompasses all time-dependent dose variables in the experiment. Dose effects are then compared on the basis of aggregate dose per irradiation cycle. (This dose/cycle is, therefore, one measure of UV radiation intensity.) For example, in the study described in the section of this chapter entitled “Repeated Exposures” (see 7.2.1.1b), the irradiation cycle equals 1 wk. (2) The *periodic dose* is defined as the smallest repeated dose quantity applied to a single group and is expressed as the product of periodic dose intensity and periodic dose duration, using consistent units of time. The periodic dose and either or both of its components may vary within an irradiation cycle.

It is useful at this point to compare the clinical and laboratory situation. Ultraviolet radiation can induce various tumor types in human skin, predominantly cell and basal cell carcinomas.⁵ Solar keratoses, carcinoma in situ, and keratoacanthomata are probably related developmentally or anatomically to squamous cell carcinoma; UV radiation apparently induces few sarcomas in human skin. The relationship of UV radiation to melanoma is still conjectural.⁶ In contrast, mice exposed to UV radiation develop essentially no distinguishable basal cell carcinomas and, as yet, a useful murine model for malignant melanoma in skin has not been developed. However, mice can develop various epithelial-derived tumors, including benign epitheliomas and carcinomas, and they can produce sarcomas under some circumstances, particularly in the skin of haired animals.^{7,8}

In mice, skin tumor types are varied but not entirely random. The distribution of tumor types can be influenced not only by skin type (haired versus hairless), but also by variables in the treatment. For example, brief exposure to dimethylbenzanthracene or UV radiation leads primarily to the formation of benign epitheliomas; more prolonged exposure increases the likelihood that carcinomas will develop.⁹ Chemical promoters can enhance the development of UV-radiation-induced papillomas or carcinomas, depending on the treatment conditions; intercurrent treatments favor carcinoma development,¹⁰ whereas sequential promotion favors papillomas.^{11,12}

7.2.1.1. Tumor Response with Dose/Irradiation Cycle as the Variable

7.2.1.1a. Single Exposure. Skin tumors can be produced in mice with a single brief exposure to UV radiation when an ulcerating dose of radiation is given.¹³ Most of the tumors produced in this way were pedunculated epitheliomas, with only 13 carcinomas among 76 mice. At a dose causing less acute damage, tumors were not produced. Follicular tumors have been produced with a single exposure to UV radiation in shaved rats.¹⁴ A single UV radiation exposure also can initiate skin tumors in two-stage carcinogenesis.

7.2.1.1b. Repeated Exposures. Most of the laboratory data on photocarcinogenesis come from studies in which the exposure is repeated from 1 to 7 times per wk for several weeks. Blum¹⁵ demonstrated the quantitative nature of the tumor response in the ears of haired mice, and we reported a similar study using body skin of hairless mice.¹⁶ The lowest periodic dose (the amount each day, 5 days/wk) was 420 J/m² (unweighted), given to group 1. The effect of increasing the amount of UV radiation per irradiation cycle is to shorten the time required to produce tumors and to increase the number of tumors produced (Figs. 7.1 and 7.2). One type of relationship demonstrable among the treatment groups is the time to 50% incidence (Fig. 7.3).

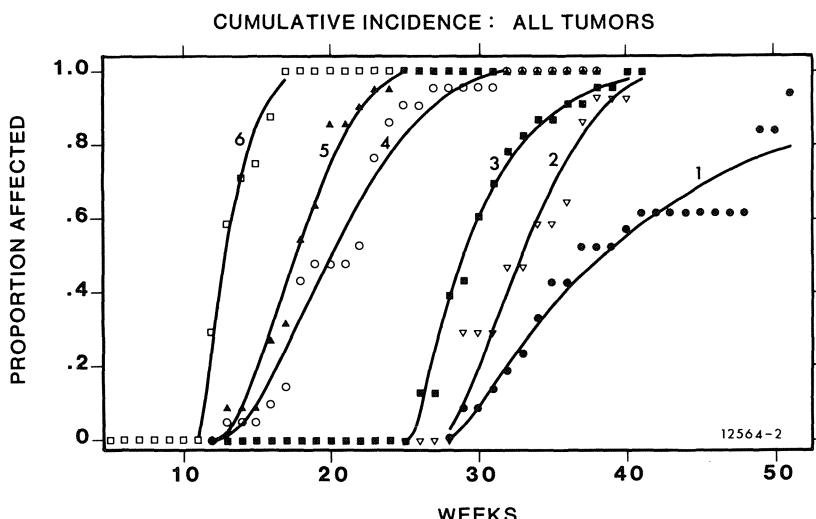


Figure 7.1. Cumulative incidence versus weeks on experiment. Groups represent 40% increments in unit dose, beginning with group 1. Minimally detectable tumors are about 0.5 mm in diameter.¹⁶ From Forbes PD, Blum HF, Davies RE: Photocarcinogenesis in hairless mice: Dose-response and the influence of dose-delivery. *Photochem Photobiol* 34:361-365, 1981, with permission.

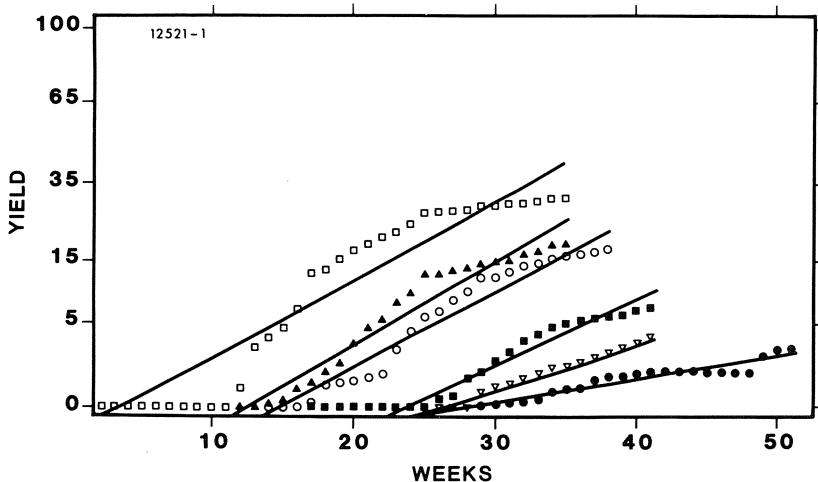


Figure 7.2. Tumor yield (average number of tumors per survivor) versus weeks on experiment (same groups as in Fig. 7.1).

7.2.1.2. Tumor Response with Dose/Irradiation Cycle as a Constant

One of the assumptions built into most of the predictions of human response to sunlight involves the concept of lifetime dose. The assumption is that the probability of developing skin cancer is directly related to accumulated lifetime dose of UV radiation, with the recognition, of course, that the contributions to the lifetime dose are made under various circumstances e.g., sunlight intensities, durations of exposure, and environmental factors. However, the

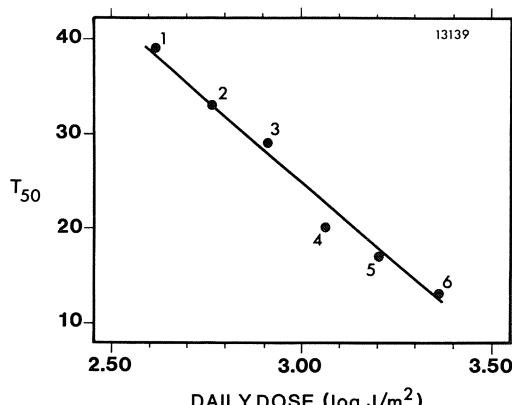


Figure 7.3. Time to reach 50% incidence (cumulative) versus daily dose of UV radiation for the 6 groups shown in Fig. 7.1.¹⁶ From Forbes PD, Blum HF, Davies RE: Photocarcinogenesis in hairless mice: Dose-response and the influence of dose-delivery. *Photochem Photobiol* 34:361-365, 1981, with permission.

inherent variables can be too numerous for interpretation of the lifetime dose. Some of these variables have been tested in mice.^{12,32}

7.2.1.2a. Daily Dose Held Constant (Irradiation Cycle Equals 1 Day). Two groups of animals were treated 5 days/wk with UV radiation to the same total dose per day. The variable was the instantaneous intensity of exposure which, therefore, determined the duration of each periodic dose. The high-intensity group was exposed for a period of 5 min, and the low-intensity group for a period of about 8 hr. The radiation delivery at low intensity was much more effective in producing tumors (P. D. Forbes, unpublished data).

Over a narrower range of time intervals (45 min-2 hr) delivery of specific integral doses can be achieved by varying either the duration or intensity of irradiation, without altering the effectiveness of the dose.¹⁷

7.2.1.2b. Weekly Dose Held Constant (Irradiation Cycle Equals 1 Wk). Three groups of animals were treated with a weekly dose, delivered either entirely on 1 day, or one-third of each of 3 days, or one-fifth on each of 5 days, chosen so that the 1-day exposure did not produce overt damage. The effectiveness of treatment increased directly with the number of fractions (periodic doses) into which the weekly dose was divided (Figs. 7.4-7.6).

Figures 7.4 to 7.6. Cumulative incidence versus weeks on experiment. All groups received identical weekly dose of UV radiation in either 1, 3, or 5 fractions (group 1 = 5 fractions, group 2 = 3 fractions, group 3 = 1 fraction). The three figures differ only in arbitrary tumor end point.

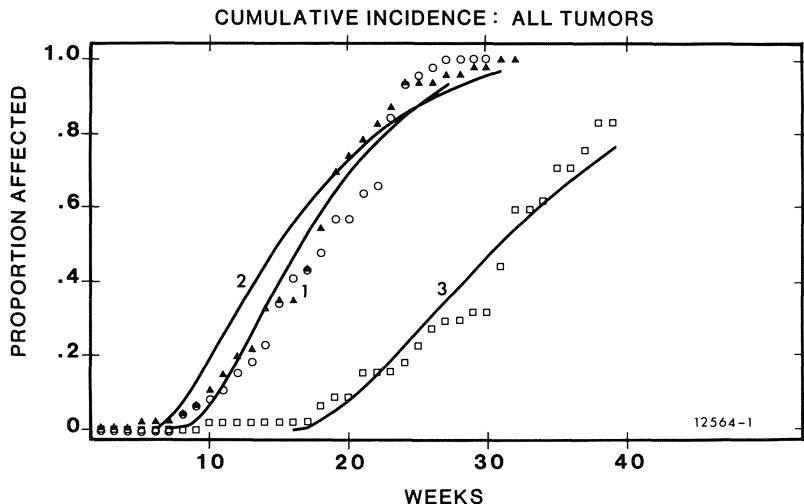


Figure 7.4. All tumors, the smallest being about 0.5 mm in diameter. From Forbes PD, Blum HF, Davies RE: Photocarcinogenesis in hairless mice: Dose-response and the influence of dose-delivery. *Photochem Photobiol* 34:361-365, 1981, with permission.

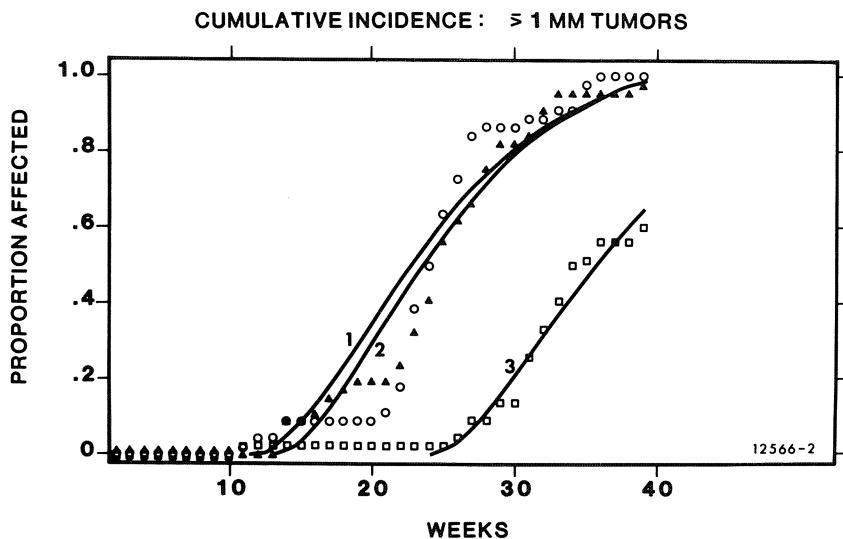


Figure 7.5. Tumors at least 1 mm in diameter. From Forbes PD, Blum HF, Davies RE: Photocarcinogenesis in hairless mice: Dose-response and the influence of dose-delivery. *Photochem Photobiol* 34:361-365, 1981, with permission.

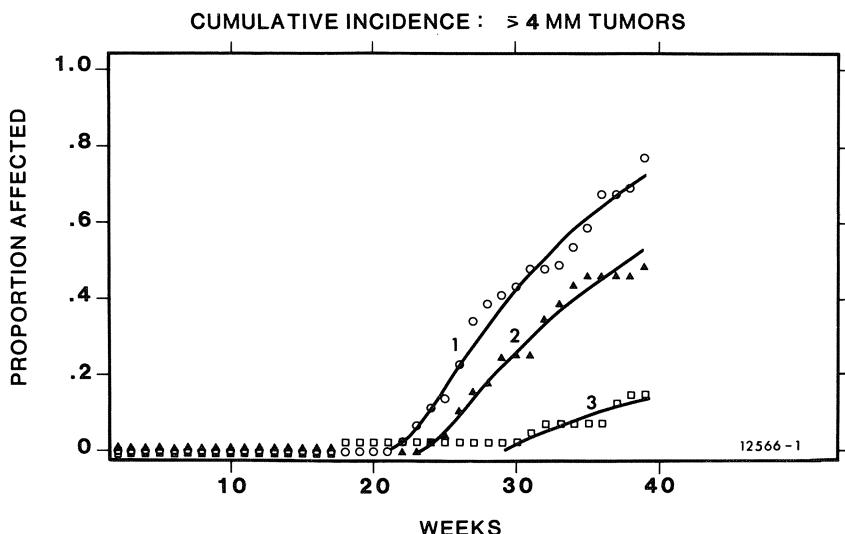


Figure 7.6. Tumors at least 4 mm in diameter.¹⁶ From Forbes PD, Blum HF, Davies RE: Photocarcinogenesis in hairless mice: Dose-response and the influence of dose-delivery. *Photochem Photobiol* 34:361-365, 1981, with permission.

7.2.1.2c. Lifetime Dose Held Constant (Irradiation Cycle Equals 10 Wk). The possible long-term consequence(s) of episodic overexposure (occasional sunburn) is an important issue in the epidemiology of human skin cancer. Our limited experience with animal studies indicates that the influence of a relatively large dose pulse is less than would be predicted on the basis of additivity alone (Fig. 7.7 and Table 7.1). In this example, periodic doses vary both within and between treatment groups.

A 10-wk course of exposures produced a significant number of tumors, however, very few of the tumors grew aggressively. We have found that continued exposure increases the likelihood that identified tumors will progress

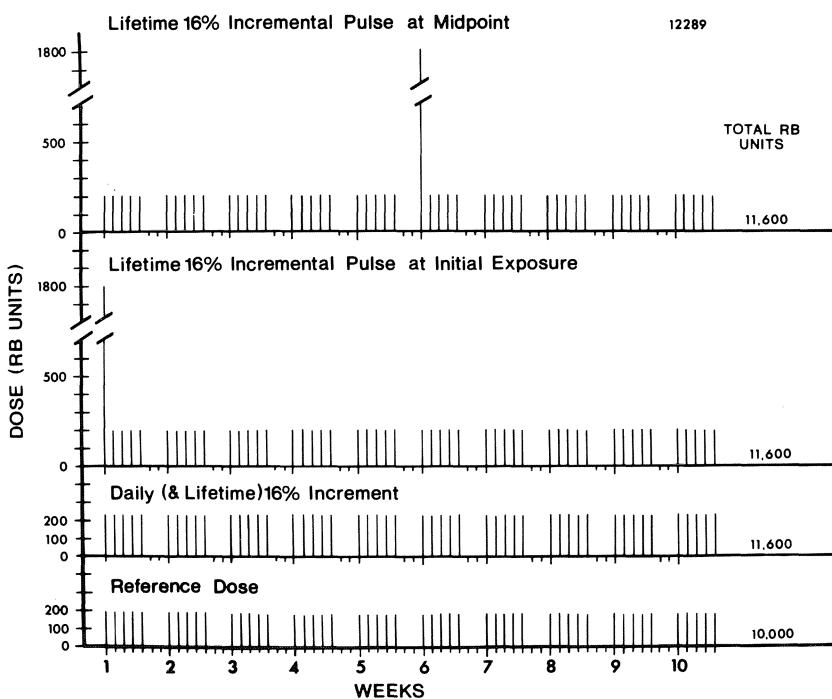


Figure 7.7. Schematic representation of experimental design: "Pulse dose" experiment. Four groups of mice were exposed to a xenon arc solar simulator, 5 days/wk for 10 wk. Group 1 received 200 RB units of UV radiation (about half the dose required to produce minimal erythema in untanned human skin) per day. Group 2 received an additional 16% daily; group 3 received its additional lifetime dose (1600 RB units) all on the first day. Group 4 received an additional 16% at the beginning of wk 6. Table 7.1 gives a summary of the results.¹⁰ From Forbes PD: Experimental photocarcinogenesis: An overview. *J Invest Dermatol* 77:139-143, 1981, with permission. © 1981, The Williams & Wilkins Co., Baltimore.

Table 7.1
Effect of the Time Distribution of an Incremental Dose of Carcinogenic UVR on the Magnitude of Response

Group	Lifetime dose (RB units)	Description	T_{50}^a	\bar{x} tumors ^b
1	10,000	Baseline dose	55	5.4
2	11,600	Daily increment	51	8.0
3	11,600	Initial pulse	66	5.0
4	11,600	Midcourse pulse	56	5.4

^a (T_{50}) Time in weeks to reach 50% incidence (median latent period).

^b (\bar{x} tumors) Average number of tumors per mouse (tumors ≥ 1 mm).

toward malignancy.¹⁸ Figs. 7.8 and 7.9 show the progression of tumor size when the tumors were exposed to UV radiation during each week of the observation period. Current hypotheses stress the initiation or promotion effects of UV radiation in carcinogenesis. One analysis of skin cancer epidemiology data favors the interpretation that UV radiation is an effective dose-related promotor of tumor growth.¹⁹ In contrast, another model indicates that tumor initiation is dose-related, whereas tumor growth is independent of the intensity of continued exposure^{20,21}

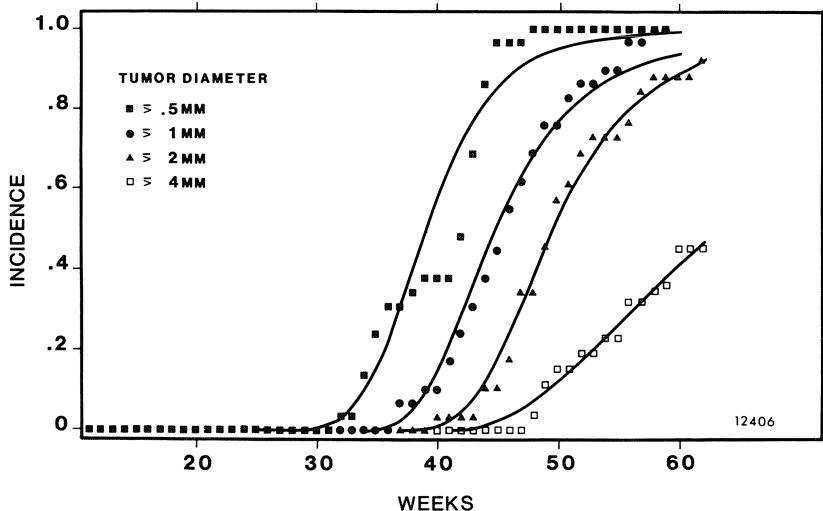


Figure 7.8. Four curves expressing incidence for one animal group, one curve for each tumor size category. The minimum size of the smallest (first-recognized) tumors is an approximation; the three larger minimum sizes are from caliper measurements. The figure shows the effect of the choice of end point criteria on apparent response. From Forbes PD, Davies RE, Urbach D, Berger D, Cole C: Simulated stratospheric oxone depletion and increased ultraviolet radiation: Effects on photocarcinogenesis in hairless mice. *Cancer Res* 42:2796-2803, 1982, with permission.

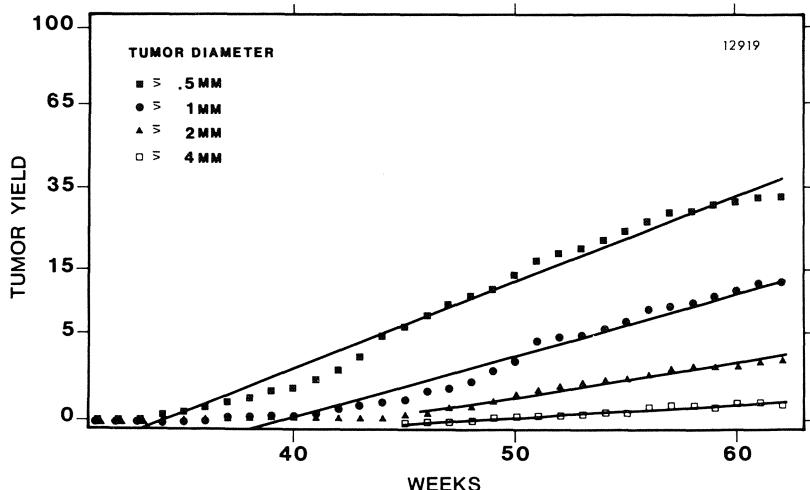


Figure 7.9. Tumor yield (average number of tumors per survivor) at each week for same group as in Fig. 7.8, one curve for each tumor size category. From Forbes PD, Davies RE, Urbach D, Berger D, Cole C: Simulated stratospheric oxone depletion and increased ultraviolet radiation: Effects on photocarcinogenesis in hairless mice. *Cancer Res* 42:2796-2803, 1982, with permission.

7.2.1.3. Amount: A Summary

Taken together, the contemporary lines of experimental data suggest that (1) within a specific dose-delivery schedule, effectiveness is proportional to dose (within limits), whether dose is varied by intensity or duration; (2) within a specific dosing schedule, effectiveness is inversely proportional to intensity and directly proportional to periodic dose duration; and (3) too little information is currently available to permit translating (1) and (2) into an equation that will accurately predict tumor response to more than a few of the possible UV radiation exposure conditions.

7.2.2. Type (Quality) of UV Radiation

The concept of action spectrum or relative efficacy of different wavelengths is well-documented with regard to the UVB region. Investigators disagree about the appropriate weighting of the spectral components of polychromatic radiation; few attempt to compare the effects of different UVB radiation sources in terms of gross (unweighted) energy. Not infrequently, however, descriptions of the inherent (detector response) or superimposed (assigned response) weighting and of the source spectrum are not sufficiently detailed to permit useful interpretation of measurements.

Investigators do not agree about the action spectrum of the UVA region.³⁵ There is little agreement on appropriate weighting(s) to be applied, and nominally

unweighted energy integrals are often presented as the simplest method of describing a treatment. Most detector systems, however, have characteristic weighting functions (action spectra); unless this has been compensated by specific calibration for the source in use, such measurements may have little value.

Furthermore, the published values for the spectral properties of a source are unreliable. These descriptions may be qualitatively useful as indicators of special precautions required for interpretation or measurement. In some cases, however, they may not even be qualitatively correct.²² For serious photocarcinogenesis experiments, there is no satisfactory substitute for reliable characterization of the source and measurement equipment.

7.2.2.1. Effective Wavelengths (Action Spectra)

Blum's classic work¹⁵ showed that the portion of the UV spectrum associated with human erythema is effective in producing skin cancer in animals. Using narrower bands of radiation, Freeman²³ suggested that the action spectrum for photocarcinogenesis in mice parallels the human erythema action spectrum at least at the wavelengths tested. The vascular responses of various mammalian species exposed to UV radiation exhibit qualitative differences^{24,25} and so it is important to make as many comparisons within one species as possible.

An exciting recent development was the demonstration that the minimal erythema threshold spectrum in man corresponds closely to acute response action spectra in hairless mice (principally for the edema response).^{26,27} These monochromatically derived spectra accurately predict the effectiveness of polychromatic sources for producing the same response (over the fairly limited series of spectral qualities tested). The spectra reasonably predicted the effectiveness of a series of polychromatic spectra for producing carcinogenesis in hairless mice. Thus, in the mouse, the rather long extrapolation from a monochromatic acute-effects action spectrum to a polychromatic chronic effect has been partially verified, lending greater credibility to the use of this extrapolation in man. Moreover, the demonstration of qualitatively and quantitatively similar acute response spectra between the two species strengthens the extrapolation of carcinogenesis studies in mice to those in humans.

7.2.2.2. Wavelength Interaction

The possibility exists that various parts of the spectrum may act in other than an additive fashion. With erythema as an end point, for example, evidence has been presented to indicate that the wavelengths longer than 320 nm either

enhance or inhibit the effectiveness of UVB radiation. Van Weelden²⁸ reported that effectiveness of an exposure to UVB radiation can be decreased by subsequently reexposing the area to UVA and visible radiation. In contrast, Willis et al.²⁹ reported that exposure to UVA radiation enhances the effectiveness of UVB radiation, particularly when the exposure to UVA radiation precedes the exposure to UVB radiation. Ying et al.³⁰ have reported that the effect is no greater than what can be attributed to additivity of effectiveness by various portions of the spectrum. Willis et al.³¹ also reported that the response to repeated UVA and UVB radiation exposures (epidermal hyperplasia and solar keratosis) has an interactive effect greater than additivity.

In our limited studies on the interaction of UVA and UVB radiation, we concluded that postexposure to UVA radiation did not enhance the long-term effectiveness of UVB radiation in producing carcinomas. For the UVB, the irradiation cycle was 10 wk. Five groups of mice were exposed to 200 J/m^2 per day, 5 days/wk for the last 2, 4, 6, 8, or 10 wk of a 10-wk period; each group was halved and replicate subgroups then received either 0 or $21 \times 10^4 \text{ J/m}^2$ UVA radiation per day, 5 days/wk for 40 wk (Fig. 7.10). An additional subgroup

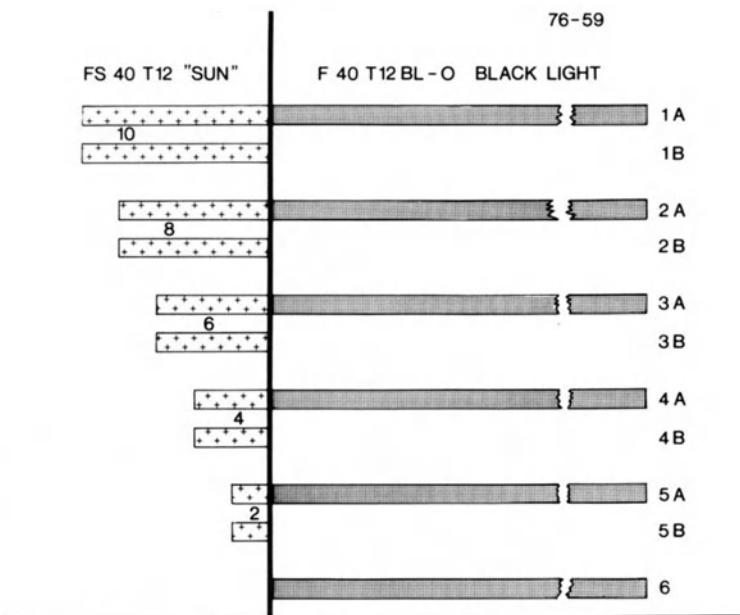


Figure 7.10. Design for experiment on the interaction of UVA and UVB radiation exposed to FS40 sunlamps for 5 min daily for 0 to 10 wk; subgroups were then left untreated (B) or exposed 6 hr daily (A) to blacklight ($\lambda > 320 \text{ nm}$).

received only the UVA radiation. For each pair of subgroups that developed tumors, the response was somewhat less in the half subsequently exposed to UVA radiation (Fig. 7.11). The possible interaction between wavelengths is an important issue that needs to be further studied.

7.2.2.3. Effects of UVA Radiation

Skin tumors have been produced in animals exposed to black light fluorescent lamps³³ perhaps because black light lamps have a measurable output in the UVB range of the spectrum.²² When these lamps are filtered with window glass, animals can be exposed 8 hr/day for several months with minimal signs of neoplasia; however, when the same daily dose is delivered at reduced intensity over a 24-hr period and the animals are exposed continuously for 10 wk, the skin of hairless mice exhibits acute and chronic changes, including the development of carcinomas³⁴ (P. D. Forbes, unpublished data). Thus, although UVA radiation is considered an inefficient producer of biologic damage, under some circumstances, it will produce many of the changes associated with exposure to UVB radiation.³⁵ We do not know whether the same biologic target is affected by the entire UV spectrum, nor whether the effects are entirely mediated by changes within the skin. There is some reason to believe that indirect effects are very significant with some types of responses.

7.3. The Influence of the Animal Model on Tumor Response

7.3.1. Anatomic Differences

The keratin of hair is a very effective scattering and absorbing medium, and consequently animals with hair are susceptible to UV-radiation-induced tumors only on the relatively naked ears, tail, and snout. Even when the hair is clipped off close to the skin surface, the hair stubble provides some protection. When the hair is chemically depilated, leaving no stubble, the sensitivity of haired mice increases significantly (P. D. Forbes, unpublished data). Whether the skin is electrically clipped or chemically depilated, haired mice tend to produce mostly sarcomas in response to exposure to UV radiation,^{7,8} whereas nonhaired mice produce primarily carcinomas.^{9,16,36}

Hairless and rhino mice have phenotypes whose controlling genes are alleles.³⁷ Both mice produce a juvenile coat of hair that is lost at the end of its growth phase; the animals do not grow a normal coat of hair subsequently. Rhino mice develop a thicker, folded skin, with a more hyperplastic epidermis and adherent stratum corneum than that of the hairless mice. Both strains of mice respond to UV radiation by producing carcinomas, but the hairless mice are significantly more susceptible. The mixed mutant allelic phenotype is distinguishable from either a hairless or rhino parent with an intermediate skin

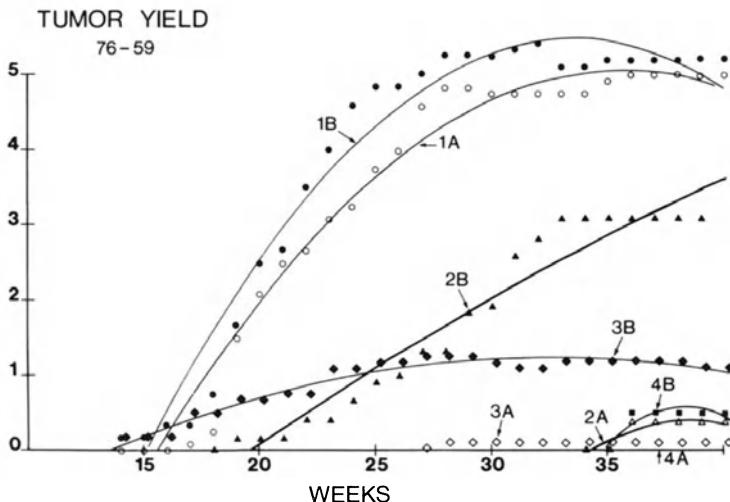


Figure 7.11. Tumor yield (tumors/survivors) from the same experiment shown in Fig. 7.10.³³ Courtesy of the NCI. From Forbes PD, Davies RE, Urbach F: Experimental ultraviolet photocarcinogenesis: Wavelength interactions and time-dose relationships. *Natl Cancer Inst Monogr* 50:31-38, 1978.

condition. We have derived homozygote hairless (hr/hr) and intermediate hairless/rhino (hr/hr^{rh}) mice with common genetic backgrounds and have exposed them to simulated sunlight.¹² The dose-response curves of these two lines of animals are similar and parallel, with a significant offset in absolute sensitivity. This is most easily seen by comparing lines representing the median latent periods for selected daily doses of UV radiation (Fig. 7.12).

Melanin pigment has long been associated with protection of human skin from damage by UV radiation. The pigmented ears of a black-haired strain of mice were shown to be less susceptible to UV-radiation-induced cancer than ears of an albino strain.³⁸ The back skin of animals with pigmented extremities is also less susceptible than the skin of albino mice, even though the skin produces very little visible pigment.³⁹ Other heritable factors may contribute to the differences in response, as will be discussed later in this chapter. The outbred hairless mouse line called Skh : HR segregates offspring whose only genetic difference, as far as we know, is at the locus affecting pigment; here again, a lowered sensitivity to UV-radiation-induced tumor formation is associated with the presence of even a very small amount of pigment in the skin.¹²

7.3.2. Differences in Tumor Response in the Absence of Anatomic Dissimilarity

We have found that two strains of albino hairless mice are significantly different in their susceptibility to tumors induced by simulated sunlight (Fig.

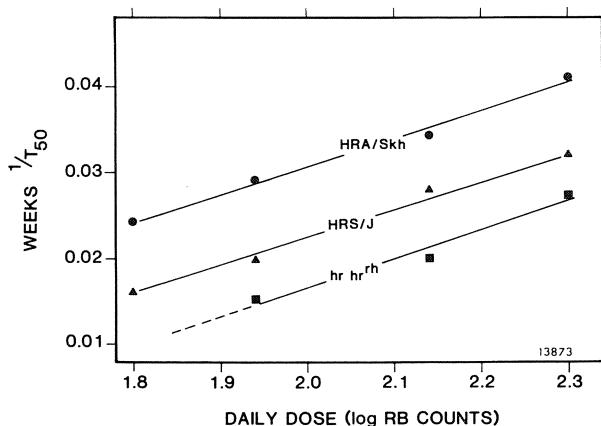


Figure 7.12. Time to produce tumors in three varieties of nonhaired mice, versus daily dose of UV radiation. The values on the ordinate scale represents reciprocals of median latent periods (50% incidence) in weeks.

7.12). Grube et al.⁴⁰ previously reported that these two strains responded differently to the carcinogenic stimulus of broad spectrum UVA radiation plus 8-methoxypsoralen. In contrast, these strains had similar dose relationships for such acute symptoms as erythema and the formation of epidermal cell thymine dimer crosslinks. We are attempting to identify the reasons for their dissimilar susceptibility to UV-radiation-induced cancer, such as subtle differences in skin structure, epidermal cell kinetics, vascular responses, or immunologic capability.

7.3.3. Effects of Age

The ear skin of haired mice⁴¹ and the dorsal skin of hairless mice⁴² become somewhat less susceptible to the formation of UV-radiation-induced tumors as age increases. Kripke and co-workers (personal communication) have found a similar change with age in the skin of clipped haired mice exposed to fluorescent sunlamps. Black et al.⁴³ have included age as a variable in a part of their studies on antioxidants. In this case, the age of the animals did not appear to influence susceptibility to UV-radiation-induced carcinogenesis.

7.3.4. Indirect Effects

One test system clearly demonstrates tumor growth under immunologic control. Ultraviolet irradiation of C3H haired mice apparently induces appearance of cells that inhibit the animals' ability to reject UV-radiation-induced tumors.^{7,44,45} Through numerous elaborate experiments, these investigators

have demonstrated the involvement of T lymphocyte suppressor cells. The system apparently can react differently to UV radiation alone or to photosensitization (psoralens plus UVA radiation).⁴⁶

de Gruijl and Van der Leun⁴⁷ have demonstrated a systemically mediated effect of UV radiation on photocarcinogenesis. Preexposing one side of hairless mice to UV radiation makes the other side more susceptible to subsequent tumor induction by UV radiation. Whether this systemic effect is immunologically mediated remains to be determined.

7.4. The Environment

7.4.1. The Chemical Environment

The inflammatory and carcinogenic properties of UV radiation have been recognized for several decades; the interaction of exogenous chemicals in photocarcinogenesis is a more recent concern and has been discussed in reviews by several authors.⁴⁸⁻⁵²

7.4.1.1. Photosensitizers

Photoinduced carcinogenesis can occur when interactive agents, which may or may not be phototoxic but in themselves are not carcinogenic, are applied to the skin before photoexposure. Among the phototoxic agents, 8-methoxypsoralen is the most widely studied in terms of photocarcinogenic interactions.^{39,48,53} The tumor response is particularly susceptible to changes in the type and amount of both drug and radiation, and in the interval between application of drug and irradiation.⁵⁴ Whether noncarcinogenic photosensitizers outside the psoralen family can also enhance photocarcinogenesis has not been studied in detail.⁵⁵

7.4.1.2. Other Chemicals That Enhance Photocarcinogenesis

Concern about the interactive effects of UV radiation actually began with the observation that UV radiation could profoundly influence chemical carcinogenesis. To generalize from a wealth of sometimes conflicting experimental data, it appears (at least in the case of mice treated with aromatic hydrocarbon carcinogens) that UV radiation may contribute in two opposing ways: by degrading the carcinogen to noncarcinogenic products and by stimulating a phototoxic response that appears to be coincident with an increased tumor yield;⁵⁶ in theory, carcinogens may also be generated photochemically *in situ*.

Croton oil promotes the growth of chemically initiated tumors; it also promotes the growth of UV-radiation-induced tumors in skin.^{10,57,58} All-trans

retinoic acid can enhance the growth of UV-radiation-induced tumors, although not necessarily by the same mechanism as croton oil. Our current studies indicate that retinoic acid, applied either between treatments with UV radiation, or applied after a course of UV radiation (promotion technique), can enhance the development of tumors and that all-trans retinoic acid in the diet can produce a similar (although reduced) effect systemically; retinoic acid can also enhance the development of tumors induced by dimethylbenzanthracene.^{11,12} Hartmann and Teelmann⁵⁹ confirm that all-trans retinoic acid does enhance photocarcinogenesis, and they report that two other metabolically active retinoids do not. Kligman and Kligman⁶⁰ failed to show that retinoic acid enhances photocarcinogenesis; whether their system was sensitive to the experimental variables tested is not clear.

7.4.1.3. Chemical Protection

Because mice fed a vitamin-A-supplemented diet develop fewer skin papillomas after a single dose of dimethylbenzanthracene than mice fed a vitamin-A-deficient diet,⁶¹ it would be surprising if retinoids under some circumstances did not offer protection against the development of skin cancer. Indeed, protection has been demonstrated under certain circumstances.⁶²⁻⁶⁶ By now it is clear only that the retinoids have a broad spectrum of biologic effects and that there are many possibilities yet to be explored.

Several antioxidants, including ascorbic acid, have been reported to reduce the effectiveness of UV radiation in producing skin cancer.^{67,68} The mechanism for this effect is not known.

Chemicals may be useful in reducing tumor risk by decreasing the quantity of UV radiation that reaches the target layer. For example, sunscreen lotion can reduce the incidence of UV-radiation-induced tumors in mouse ears⁶⁹ and on the backs of hairless mice.⁷⁰

7.4.2. The Physical Environment

7.4.2.1. Heat, Humidity, Wind

An increase in susceptibility to UV-radiation-induced tumors has been linked with increased room air temperature, relative humidity, and wind speed.⁷¹ The incidence of mouse mammary tumor is influenced by room fluorescent lighting.⁷²

7.4.2.2. Other Radiations

Little is known about the possible interactive effects of ionizing radiation and UV radiation. Some early reports, based primarily on clinical impressions,

suggested these effects were more than additive.^{73,74} Previous radiation therapy is one of the variables suspected in the increased susceptibility to carcinoma of patients treated with 8-methoxypsoralen plus UVA radiation.⁷⁵ The possible influence of microwave irradiation in influencing UV-radiation-induced photocarcinogenesis has not yet been studied.

7.5. Conclusion

Studies on carcinogenesis in humans are primarily retrospective; with notable exceptions, such studies have few quantitative data available on the delivery of the putative carcinogen. The theoretical advantages of laboratory animal studies include control over the variables to be tested, such as the choice of test agent, and the amount or mode of exposure. Ideally, the test will be conducted in a system whose range of response is known; in a given study, the positive control is chosen to demonstrate that the test system is capable of providing a measurable response. Not all experimental designs have taken advantage of this concept. The inherent disadvantage of laboratory studies is that clinical extrapolations depend on the validity of the experimental model only certain attributes of which have been examined thoroughly.

With these limitations in mind, the evidence indicates that photocarcinogenesis in skin is influenced significantly by the spectral character of UV radiation, by the integrated dose, by the mode of its delivery, and by a number of interactive factors; these factors include heritable differences in tumor susceptibility, some immunologic responses, and numerous chemicals. Our understanding of some of these factors is better than of others; we anticipate a rapid growth of information in this field.

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Section II

Experimental Photoimmunology

Historically, photobiology and immunology have been combined in experimental investigations primarily in the context of clinical dermatology. A study of the effects of nonionizing radiation on the cutaneous manifestations of immunologic reactions is a natural path of inquiry in dermatologic research. However, in the past few years, photoimmunology has moved beyond its clinical cutaneous focus to include the study of basic mechanisms in immunology, cell biology, and carcinogenesis. It is this new growth in photoimmunology that is responsible for its aura of excitement and anticipation.

Until recently, photoimmunology consisted of isolated observations that had no obvious interrelationships. In fact, a survey of current work in this area shows clearly that many of today's key observations have existed undisturbed in the literature for many years and thus represent only rediscoveries of findings that were made by others in the past. What has changed this picture in recent years is the establishment of a few vital links between immunology and skin architecture and between immunology and UV radiation. From these connections, a cohesive picture of the interactions among light, immunologic reactions, and skin has begun to emerge. The realization of these interactions has catalyzed a flurry of activity in this field, and there is a strong sense among investigators that many of the isolated and unexplained observations are about to fall into place. In addition, momentum has been generated by the possibility that the study of photoimmunology will increase our understanding of important and fundamental principles in human biology and immunology and that therapeutic applications of these principles will follow the experimental observations.

An accurate perspective during a time of rapid progress is difficult to maintain. Therefore, the significance of many experimental observations and the interpretation of many findings must remain speculative. Furthermore, since this is presently an active, dynamic field of investigation, much of our speculation will be obsolete within a short period of time. Given these circumstances, we will review the historical development of this field, will summarize its current

state of knowledge, and, most importantly, will provide at least the beginnings of a framework for understanding it. We hope to underscore the reasons for the present interest and activity in this field of research and to convey an appreciation for the potential of photobiology as an approach to analyzing the complexities of the immune system and its intimate relationship with the skin.

M.L.K.

Chapter 8

Immunobiology of UV-Radiation-Induced Skin Cancer

Margaret L. Kripke

8.1. Experimental Studies in Mice

Nearly all of the recent studies on the immunology of UV radiation-induced tumors and many of those addressing the immunologic consequences of exposure to UV radiation can be traced back to the simple observation that UV-radiation-induced skin cancers in mice are difficult to transplant. This observation was reported first by Rusch and Baumann in 1939,¹ and was rediscovered by Graffi and his associates in 1964 during their studies of the antigenicity of these tumors.^{2,3} In both instances, it was considered as nothing more than a curious and annoying feature of photocarcinogenesis in the mouse. In 1974, the observation was made for the third time by Kripke,⁴ but this time the phenomenon was so striking that it could not be dismissed as trivial. In this study, most of the UV-radiation-induced skin cancers could not be propagated by transplantation to genetically identical (syngeneic) mice. Investigation of this unusual biologic behavior revealed that the failure of these tumors to grow in normal syngeneic mice was caused by immunologic rejection. This discovery was made possible by the fortuitous choice for these experiments of inbred strains of mice that are particularly good immunologic responders. As a consequence, these animals were able to completely reject a large proportion of the UV-radiation-induced skin tumors.^{4,5}

These are two types of evidence that the regression of these tumors is mediated immunologically. First, although the tumors regress in normal mice, they will grow progressively in mice that are immunosuppressed in various ways, including antilymphocyte serum treatment, X-irradiation, and congenital athymia.⁴⁻⁶ Second, tumor regression in normal animals is accompanied by the development of specific immunity, or heightened resistance, to the implanted

tumor. This immunity can be measured in various ways. *In vivo*, mice in which a tumor implant has regressed can be given a sublethal dose of X rays that is sufficient to eliminate a primary immune response but that is insufficient to affect immunologic memory. Upon challenge, the tumor is rejected only by animals that were exposed previously to that particular tumor.⁴ Or, lymphocytes that are specifically cytotoxic for the tumor cells *in vitro*⁷ can be taken from mice in which a tumor transplant has regressed; these lymphocytes will passively transfer specific immunity to immunodeficient animals.⁸ These studies demonstrate that the regression of the UV-radiation-induced tumors is caused by immunologic rejection of the tumor transplants. Furthermore, they confirm the original finding by Pasternak et al.³ that these tumors induced by UV radiation, like those induced by chemical carcinogens, have individual, or noncrossreactive, tumor-specific transplantation antigens.

However, all UV-radiation-induced tumors do not regress after transplantation of tumor fragments. The proportion of regressor tumors produced with a given regimen of exposure to UV radiation differs among different mouse strains⁵ probably because of differences in their immunologic capabilities. These differences are the likely reason that the regression phenomenon went unremarked until investigators used C3H mice, in which the proportion of regressor tumors is high, to produce tumors with UV radiation. However, even the progressor tumors, which grow in normal recipients, are highly immunogenic relative to tumors induced by other carcinogens.^{9,10} Titration of the tumor cells in normal and immunosuppressed mice generally will reveal a dose of cells that grows in the immunosuppressed mice but not in the normal recipients.

At the present time, little is known about how these tumors are rejected by normal recipients and about which effector mechanisms participate in the regression process. The tumors grow in T-lymphocyte-depleted mice^{4–6,11} and the transfer of immunity is effected by T lymphocytes,⁹ indicating that regression is a T-lymphocyte-mediated reaction. However, this finding does not prove that the ultimate effector cells are T lymphocytes. T lymphocytes play a regulatory role in the production of antibody by B lymphocytes and produce soluble factors that activate and recruit other cells that can destroy tumor cells, in addition to acting as cytotoxic effector cells; again, however, these findings do not prove that the T lymphocytes are involved directly in tumor cell killing *in vivo*. Circumstantial evidence for the participation of cytotoxic T lymphocytes in tumor regression has been provided by Lill and Fortner, however.¹² They were able to recover the host cells infiltrating a tumor during its regression in normal recipients. The infiltrates were composed of mainly T lymphocytes and polymorphonuclear leukocytes, with a smaller proportion of macrophages, and few B lymphocytes. The T lymphocytes were highly cytotoxic for the specific tumor *in vitro*, even at effector-to-target-cell ratios as low as 1:1. The highest number of cytotoxic T lymphocytes was present between 4 and 6 days

after tumor implantation, at a time just preceding the morphologic degeneration of the tumor tissue. This association, along with the absence of cytotoxic T lymphocytes in tumors that do not regress, suggests that cytotoxic T lymphocytes comprise at least one effector mechanism in the regression of these tumors. Lymphocytes from tumor-immune mice produce soluble factors in culture that specifically activate macrophages to kill tumor cells *in vitro*.¹³ This observation, coupled with the finding that tumor rejection is impaired in macrophage-depleted mice,^{14,15} suggests that this immunologic pathway also may be important in tumor regression *in vivo*.

These findings led to the conclusion that UV-radiation-induced murine tumors have an unusually high degree of antigenicity, and they raised the question of how these tumors could survive immunologic rejection in the primary host. Investigations directed to this question provided one of the major links between immunology and UV radiation by demonstrating that treatment of mice with UV radiation interfered with the immunologic rejection of UV-radiation-induced tumors at distant sites.¹⁷ Transplantation of UV-radiation-induced tumors into UV-irradiated mice resulted in progressive growth of the tumor transplants, rather than rejection. This susceptibility to tumor transplantation occurred early in the course of UV irradiation, before primary tumors were evident. Intravenous injection of tumor cells resulted in the development of pulmonary and extrapulmonary metastases in UV-irradiated, but not unirradiated, animals.¹⁸

Recent experiments on the photobiologic aspects of this susceptibility to transplanted tumors have demonstrated that the dose of UV radiation from FS40 sunlamps required to induce susceptibility is directly related to the \log_{10} dose of UV radiation, and unlike carcinogenesis, its induction exhibits reciprocity with regard to time and dose of radiation. The active waveband in the sunlamps for the induction of tumor susceptibility in albino mice appears to be between 275 and 315 nm, based on experiments using plastic filters.^{19,20}

The immunologic nature of this inability to reject UV-radiation-induced skin cancers was established in a series of experiments performed by Fisher and Kripke.²¹ One approach involved reconstitution of lethally X-irradiated mice by an intravenous injection of lymphoid cells from UV-irradiated or untreated donors and then challenge of these animals with a transplant of a UV-radiation induced tumor. Mice that received normal lymphoid cells could reject the tumor transplant, but those reconstituted with lymphoid cells from UV-irradiated donors were susceptible to tumor growth. This indicated that the inability of UV-irradiated mice to reject the tumor implants was caused by an alteration in their lymphoid cells. Fisher and Kripke also demonstrated that T lymphocytes that prevented tumor rejection were present in the altered lymphoid cell population. They reconstituted lethally X-irradiated mice with a mixture of lymphoid cells from normal and UV-irradiated mice and found that these animals were

susceptible to tumor challenge.²¹ Subsequent studies demonstrated that T lymphocytes from the UV-irradiated mice suppressed tumor rejection by the normal lymphocytes.²² Using a different approach, Spellman and Daynes also demonstrated that lymphoid cells from UV-irradiated mice contained suppressive activity.²³ They injected lymphoid cells from UV-irradiated donors intravenously into normal recipients and found that the recipients were rendered susceptible to tumor challenge. Furthermore, they showed that removal of the T lymphocytes from this lymphoid cell preparation removed its suppressive activity.²⁴

These suppressor lymphoid cells are part of a regulatory system that normally controls immunologic responses. The suppressor cells induced by UV radiation inhibit the immune response against UV-radiation-induced skin cancers in the UV-irradiated host in the absence of any other antigenic stimulus. The cells are specific in that only the immune response against syngeneic UV-radiation-induced tumors appears to be suppressed. The rejection of tissue allografts and chemically induced syngeneic tumors is unaffected by these suppressor cells.^{10,21,22} Attempts to characterize these suppressor cells further and to determine their mode of action have been hampered by the lack of an *in vitro* assay with which to measure their activity. *In vivo* studies have demonstrated that these cells are present in mice long after the cessation of UV irradiation,²³ that they are eliminated by X-irradiation of the host, and that they appear to express an Ia surface determinant.⁹ The cells do not appear to act by stimulating tumor growth directly.¹⁰ These cells will suppress tumor rejection when mixed with normal lymphocytes and tumor cells and injected subcutaneously into immunosuppressed recipients,²⁵ but they do not inhibit the rejection of tumors by immune lymphocytes, either subcutaneously (J. Mattes, unpublished data) or systemically.^{8,9} These results suggest that the suppressor cells act at an early stage in the generation of the immune response against the tumors and not by direct inhibition of the effector cells.

Using an assay that measured the cytotoxicity of T lymphocytes against cultured tumor cell lines, Thorn¹⁶ and Thorn et al.²⁵ attempted to determine the site of action of the UV-radiation-induced suppressor cells. He found that primary sensitization either *in vitro* or *in vivo* against UV radiation-induced fibrosarcoma cells occurred equally well with UV-irradiated and normal animals. The addition of UV-radiation-induced suppressor cells *in vitro* to various stages of the cytotoxic reaction, from the generation of primary cytotoxic cells to the killing of target cells by immune lymphocytes, had no effect on cytotoxic activity. However, lymphocytes from UV-irradiated mice that had been immunized, produced less cytotoxic activity when restimulated *in vitro* than lymphocytes obtained from unirradiated donors. Based on these results, Thorn concluded that UV-irradiated mice are deficient in their cytotoxic memory response against UV-radiation-induced tumors and that the UV-radiation-induced suppressor cells

function by inhibiting the generation of cytotoxic memory cells. This method for determining inhibition requires *in vivo* immunization of animals containing the suppressor cells and, therefore, does not fill the need for a simple *in vitro* assay for the activity of these suppressor cells. The interpretation of these results is limited by the probability that the cytotoxic T lymphocyte is not the only effector cell involved in tumor rejection. Thus, the validity of any conclusions drawn from them depends upon how accurately the cytotoxic T cell response represents the process of tumor rejection *in vivo*.

The way in which the UV-radiation-induced systemic alteration and the UV-radiation-induced suppressor cells affect the appearance of primary skin cancers has been examined recently. De Gruijl and Van der Leun²⁶ have shown that photocarcinogenesis in hairless mice is accelerated in animals that have been exposed previously to UV radiation at a separate site. This finding supports the concept that the systemic effects of UV radiation treatment can influence the development of primary skin cancers and control the growth of UV-radiation-induced tumor transplants. A report by Fisher and Kripke²⁷ indicates further that the presence or absence of UV-radiation-induced suppressor T lymphocytes can dictate whether or not visible tumors will develop in UV-irradiated skin. Both studies support the view that the long latent period for the appearance of these skin cancers is caused in part by immunologic elimination of nascent tumors that arise early in the course of irradiation. Studies demonstrating that UV-radiation-induced skin cancer appears earlier in mice treated with certain immunosuppressive regimens than in untreated mice also are consistent with this interpretation.^{11,15,28}

8.2. Tumor Antigens

8.2.1. General Considerations

The antigens on the tumor cells that are responsible for the rejection of UV-radiation-induced skin tumors are called either tumor rejection antigens or tumor-specific transplantation antigens (TSTA). They result from a genetic alteration, rather than from a direct effect of UV radiation on the cell membrane, as evidenced by the fact that antigenic changes are heritable. Passage of the tumors in immunosuppressed mice or in cell culture for several generations does not usually result in the loss of the antigens. The antigens do not only occur in cancers induced by UV radiation, but also are common in cancers induced experimentally by chemical carcinogens, oncongenic viruses, and ionizing radiation.

In the broadest sense, a tumor antigen is any molecule on the surface of a tumor cell that can induce an immune response in the host. There are many

examples of inappropriate or abnormal cell membrane components that are expressed by neoplastic cells and, thus, can be considered tumor-associated or tumor-specific antigens, such as viral proteins, fetal antigens, or secreted cell products. However, the most important tumor antigens from the immunologic point of view are those that stimulate a tumor rejection reaction. These TSTA are present on tumor cells, and not on normal cells, of the same histologic type and are defined and detected by their ability to induce protective immunity against transplanted tumor cells. The classical test for TSTA is to immunize groups of animals by implanting viable tumor tissue and by excising the resulting tumors surgically, and then to challenge the animals at a different site with a dose of tumor cells calculated to produce tumors in about 80% of nonimmunized recipients. A significant reduction in tumor incidence or rate of tumor development is presumptive evidence that immunization has occurred and that TSTA are present on the immunizing tumor. Formal proof that this tumor resistance is mediated by immune response against TSTA can be obtained by testing the specificity of the resistance for the immunizing tumor and by transferring the resistance to a secondary host with lymphocytes from the immunized animals. These tests for TSTA can be performed only with inbred animals. Members of an inbred strain have a high probability of being homozygous at all genetic loci and of being genetically identical with each other. This permits transplantation of tissues among members of a single strain because the tissues are not recognized as foreign. Only under these conditions can the presence of TSTA be established.

The antigenic targets of the immune reaction probably are located on the surface of the tumor cells. New antigens that are present inside the tumor cell are inaccessible to the immune system and are, thus, unlikely to play a role in the rejection of viable tumor cells. The nature and function of TSTA are a matter of great speculation. In the case of some virus-induced tumors, the TSTA are new antigens that are encoded by the viral genome. In this case, all cells transformed by a particular virus, regardless of their histologic type, share the same TSTA. Among chemically induced tumors, however, each TSTA is unique; that is, immunization with one tumor is protective against itself, and not against other tumors. Even two tumors of the same histologic type, induced by the same carcinogen in a single animal, will not exhibit crossreactive antigens. A second distinctive feature of the antigens of chemically induced tumors is the lack of uniformity in the degree of antigenicity that they exhibit. With any given carcinogen, some tumors produced will be highly antigenic (i.e., induce a high level of protective immunity), whereas other tumors will evoke little or no protective immunity. This range in immunogenicity is characteristic of tumors induced by all chemical carcinogens that have been studied; however, a particular carcinogen may tend to induce tumors that, as a group, are more or less antigenic than those induced by another carcinogen.^{29,30}

The basis for these two types of diversity, individual specificity and antigenic strength, is unknown. All we know is that these properties are characteristic of the transforming event and not of the particular cell that is being transformed. This was demonstrated by transforming with a chemical carcinogen many cells that were the recent descendants of a single progenitor. All of the resulting tumors were individually specific, even though they originated from the descendants of the same cell.³¹ Once transformation has occurred, the antigenic change appears to be stably associated with the transformed phenotype. The most striking example of this association is the recent study by Hopkins and Law³² which showed that transfer of the neoplastic phenotype to normal recipient cells using DNA fragments isolated from tumor cells was associated in most cases with transfer of the TSTA from the donor tumor. This suggests that the TSTA are an integral part of the transformation process and are associated with it at the genetic level; however, their role in transformation remains to be elucidated.

8.2.2. Antigens of UV-Radiation-Induced Tumors

As mentioned earlier, the first studies on the antigenicity of UV-radiation-induced skin cancers were performed in the 1960s by Graffi and his colleagues.^{2,3} Using standard *in vivo* transplantation tests, these investigators found that UV-radiation-induced sarcomas from strain XVII mice were highly antigenic compared to sarcomas induced by other chemical and physical agents and that these tumors had individually specific antigens.³³ These findings have been confirmed using both sarcomas and squamous carcinomas from several different mouse strains.^{4,5} Thus, skin cancers induced in mice by UV radiation resemble those induced by chemical carcinogens in that each tumor can immunize against itself, but cannot immunize against other UV-radiation-induced tumors.

Induction of immunity by standard transplantation techniques evokes a cell-mediated immune response that is specific for the immunizing tumor. *In vitro* assays of this immune response, using lymphocytes from tumor-immune mice and cultured tumor target cells generally detect individually specific antigens also.^{7,16,34} However, Werner et al.³⁵ reported the detection of both unique and crossreactive antigens on UV-radiation-induced sarcomas, using the decreased electrophoretic mobility of lymph node cells from tumor-bearing mice in the presence of soluble tumor cell membrane extracts as a measure of immunity. In addition, studies by Daynes and co-workers³⁶ indicated that immunization of mice with UV-radiation-induced tumors results in the production of cytotoxic lymphocytes, some of which recognize individually specific antigenic determinants and others that recognize common determinants. This group also has reported that hyperimmunization of mice with a UV-radiation-induced

tumor induces crossprotective transplantation immunity,³⁷ however, this report of crossreactivity in an in vivo rejection test has not been confirmed.

Studies of cell-mediated immunity and tumor rejection in vivo make it clear that UV-radiation-induced tumors have individually specific TSTA. What is less certain is whether these tumors also express common antigenic determinants that can contribute to tumor rejection. The existence of such common antigens is suggested in the studies of UV-radiation-induced suppressor lymphocytes. Although tumor immunity is specific for the immunizing tumor, the suppressor cells prevent the rejection of many different UV-induced tumors, but not tumors of other etiologies. This raises the interesting and important question of how it is possible to suppress the immune response specifically against a set of apparently noncrossreacting antigens. One possibility is that the suppressor cells are directed against a common antigen that is expressed on all tumors induced by UV radiation, whereas the effector cells are directed mainly against the individual antigen.³⁷ Another possibility is that the tumor antigens represent a family of chemically related molecules composed of both common and individually specific determinants. In this model, the immunologic regulatory cells would be directed against the common determinants but would regulate the activity of effector lymphoid cells that were directed against the individual specificities.³⁸ A third possibility is that the UV-radiation-induced tumors possess only individually specific TSTA and that the suppressor cells comprise a heterogeneous population of multiple clones, each of which is specific for a single tumor. In this case, the suppressor cells induced during UV irradiation would prevent the rejection of all UV-radiation-induced tumors, but individual suppressor cells would be specific for only a single tumor.

This question of the specificity of immunologic regulation is not merely of academic interest, its answer may provide new insights and approaches for controlling the immune response against autologous tumors. If the regulatory cells of the immune system are directed against common antigens or determinants, and not against the same individual specificities as the effector cells, then immunotherapy could be directed toward the regulatory network of the immune system, instead of toward the effector components. Thus, it is important to determine whether UV-radiation-induced tumors have common antigenic determinants, in addition to the individually specific ones. One approach that has been used to address this question is to attempt to elicit antibodies against the tumors and to analyze the specificity of their binding to the tumor cells. Since the UV-radiation-induced tumors are so effective in inducing cell-mediated immunity, one might expect that they would readily elicit the formation of antibodies. However, this has not turned out to be the case. Although it has been possible to detect antibodies against UV-radiation-induced tumors,^{39,40} the antibodies appear to be of low affinity and present in low concentration in immune sera. The antisera that have been raised against these tumors exhibit extensive

crossreactions, many of which can be attributed to antibodies with specificity for antigens of murine leukemia viruses that are carried endogenously within the tumor cells.³⁹ By removing these antiviral antibodies, or by using the antibodies that arise early in the course of immunization, it is possible to demonstrate cytotoxic activity or binding activity that is specific for an individual tumor. However, the activity is low, and thus, this tumor system has not provided much of an advantage over the chemically induced tumor systems that have been studied previously.

The detraction of this serological approach to analyzing tumor antigens is the difficulty of proving that the antigens detected by the antisera are the same as those responsible for tumor rejection *in vivo*. The attraction of the approach, of course, is the potential for using antibodies to isolate and purify tumor antigens in order to elucidate their chemical composition. To date, this approach has been unsuccessful, but with the advent of monoclonal antibodies, the problems of heterogeneous sera with specific antibodies of low titer may be obviated. However, the problem of relating antigens detected by antibodies to the antigens responsible for tumor rejection still must be resolved. Thus, the conclusions that can be drawn from these studies regarding antigenic specificity are that antibodies can detect both individually specific determinants and shared determinants on UV-radiation-induced tumors, but the relationship of these antigens to those involved in tumor rejection is unknown.

The direct approach to understanding the antigenic relationships among UV-radiation-induced tumors is to isolate and purify these antigens and determine their molecular structure. Preliminary attempts to do this have been reported^{41,42} and, in both cases, induction of tumor immunity *in vivo* was used as the assay to monitor antigen isolation, rather than relying on antibodies for purification. Ransom et al.⁴² have been able to isolate a 76,000-dalton glycoprotein from a UV-radiation-induced sarcoma that induces immunity against this tumor, but not against a second UV-radiation-induced sarcoma from the same mouse strain. Thus, it should be possible eventually to compare the structure of several such antigens, to assess their degree of similarity, and to determine whether the UV-radiation-induced suppressor cells recognize this glycoprotein.

8.2.3. Comparisons with Other Tumors

There are two interesting questions that arise from the studies of TSTA in the UV radiation carcinogenesis system: (1) Why are these tumors so highly antigenic compared to tumors induced by chemical carcinogens? and (2) Do the antigens on UV-radiation-induced tumors have something in common as a group that distinguishes them from tumors induced by other agents? With regard to the degree of antigenicity, one might ask whether tumors induced by other types of radiation or by UV radiation combined with sensitizing chemicals exhibit this

unusual property. Thus far, studies with tumors induced by psoralen plus UVA radiation⁴³ and those induced by 4-nitroquinoline oxide (M.S. Fisher, unpublished data) indicate that these tumors are not highly antigenic, and in this respect differ from those induced by UVB radiation. However, these studies cannot be considered definitive as yet, because it is possible that these agents produce highly antigenic tumors but that the tumors are immunologically rejected in the primary host. Thus, it is necessary to determine the degree of antigenicity of tumors that are induced by these agents *in vitro* or in animals that are immunologically deficient. In this regard, studies have shown that tumors induced with polycyclic hydrocarbon carcinogens in mice that have been immunosuppressed by UV radiation are more antigenic as a group than are tumors induced in unirradiated animals.⁴⁴

Tumors also have been produced by UV irradiation of murine epidermal cells⁴⁵ and fibroblasts^{46,47} *in vitro*. These tumors may be highly antigenic like those induced *in vivo* by UV radiation.⁴⁵ However, a sufficient number of comparisons with tumors induced *in vitro* by agents other than UVB radiation has not yet been carried out to establish this point with certainty. Preliminary evidence suggests that tumors transformed *in vitro* with either UVB radiation or UVC radiation are recognized by UV-irradiated recipient mice as belonging to the set of UVR-induced tumors¹⁰ (M. Fisher, G. Chan, M. Kripke, unpublished data). These preliminary findings, coupled with the finding that the UV-irradiated host can discriminate between UVB-radiation-induced and psoralen-plus-UVA-radiation-induced tumors,⁴³ suggest that antigens of UV-radiation-induced tumors might not represent random molecular alterations, as has been supposed in the past. Rather, they might be related in some orderly and fundamental manner, to their etiologic agent, even though they appear to be antigenically unique in transplantation tests. The fact that these tumors appear to be recognized immunologically as a distinctive class by the UV-irradiated host implies such an association between antigenicity and etiology, at least in the case of UV-radiation-induced tumors. Whether this hypothesis proves to be correct or not, the UV-radiation-induced tumor system is providing new insights and approaches to the questions of the nature and significance of tumor antigens.

8.3. Significance of the Experimental Studies

The findings summarized above indicate that there is an important immunologic component to photocarcinogenesis in the mouse. Thus, this tumor system is an excellent model in its own right for defining the contributions of host immunologic mechanisms to the development and pathogenesis of cancer. It represents the only nonviral experimental model for carcinogenesis in which the host immune response to the tumors has been studied during the period before cancers can be detected and in which there is convincing evidence for the

existence of an immunologic surveillance system against developing cancers. A certain minimal level of antigenicity probably is required to trigger immunologic recognition of endogenous tumor cells. Ultraviolet-radiation-induced tumors, which are more highly antigenic than the chemically induced tumors, must exceed this minimum. Moreover, the development of these highly antigenic tumors in the skin, rather than in another organ, may be a critical factor because of the intimate and unique association between the immune system and the integument. The tumor model also clearly shows that immunologic studies performed on tumors transplanted into normal animals do not necessarily reflect the situation within the primary host. In the photocarcinogenesis system, the tumors grow progressively in their original host, but they are rejected immunologically when they are transplanted into normal recipients. In this instance, investigating the rejection of transplanted tumors is not likely to provide information on how to control tumor growth by immunologic means in the primary host. Exposure of the host to a carcinogenic agent may also have systemic effects that influence the host response against tumors that arise subsequently. The model underscores the importance of immunologic regulatory mechanisms in controlling cancer development. At present, it represents the only experimental system in which it has been demonstrated that the immunologic regulatory pathway can play a determining role in carcinogenesis.

The finding that suppressor T cells in UV-irradiated mice inhibit tumor rejection has provided a new system with which to investigate how these regulatory cells suppress the immune response. Little is known about the details of how tumors are rejected immunologically and of the mechanisms that control the rejection response. The photocarcinogenesis model has provided new material and new approaches for studying these issues. As mentioned earlier, one important question that is raised by these studies concerns the specificity of immunologic regulation. Each of the tumors induced by UV radiation has a unique TSTA, such that immunization with one tumor confers protection against only that tumor and not against other UV-radiation-induced tumors.^{3,4} The fact that the UV-radiation-induced suppressor cells inhibit the rejection of all of these tumors seems paradoxical in that the suppressor cells appear to be "specific" for a set of non-cross-reacting antigens. This situation raises the possibility that the specificity of the regulatory part of the immune response may be different from the specificity of the effector cells. This is an important issue in trying to understand how immunologic regulation occurs, and it has broad implications for the design of approaches for manipulating the immune response in order to destroy tumor cells *in vivo*. Thus, studies of this tumor system have the potential to provide new insights into the mechanisms of immunologic regulation, and they also may lead to more effective approaches to the immunologic control of cancer.

In addition to providing a link between skin cancer and the immune system, these studies have also established a connection between UV radiation and the

immune system. One of the most important, and perhaps most unexpected, findings is that superficial exposure of mice to UV radiation has systemic, immunologic consequences important in the pathogenesis of UV-radiation-induced skin cancer. This finding has stimulated a renewed interest in the effects of UV-radiation exposure on cutaneous immune reactions and in the effects of UV radiation on systemic as well as local immune responses. In addition, the finding of suppressor lymphocytes in UV-irradiated mice has opened another avenue of investigation that addresses the question of how UV radiation can initiate this specific immunologic effect.

8.4. Studies on Human Skin Cancer

Although a strong argument can be made for a major role of the immune system in some experimental models of photocarcinogenesis, evidence for the participation of immunologic mechanisms in the development of human skin cancer is less certain. The most convincing support for this possibility derives from studies on the incidence of sunlight-associated skin cancer in the recipients of renal transplants. These patients, who receive long-term immunosuppressive therapy to prevent rejection of their grafted kidneys, have a higher risk of developing skin cancer, particularly squamous carcinoma, on sites of the body exposed to the sun.⁴⁸⁻⁵² In addition, there are reported instances of rapid skin cancer development in patients treated with immunosuppressive chemotherapeutic agents for lymphoid malignancies.^{53,54} Although these results are consistent with the view that immunologic factors are important in human photocarcinogenesis, they do not constitute proof of this hypothesis. There is no assurance that it is the immunosuppressive properties of these drugs that is affecting tumor development, since all have a wide variety of effects on host tissue in addition to their immunosuppressive action. Furthermore, it is not certain that these skin cancers are induced by sunlight alone; human papilloma virus may be involved also.

A study by Dellon et al.⁵⁵ provides a second line of evidence that suggests an association between the immune system and skin cancer. These investigators analyzed the number of T lymphocytes in the peripheral blood of 75 patients with skin cancer and correlated this value with tumor size and the degree of lymphocytic infiltration in the tumor. They found that patients with squamous or basal cell carcinoma, particularly those with large tumors, had fewer circulating T lymphocytes than normal subjects without cancers. In addition, small localized lesions tended to have more extensive lymphocytic infiltrates than did large, invasive tumors. Attempts to identify precisely the mononuclear cells infiltrating cutaneous tumors have been made by Thivolet, Bustamante, and co-workers.^{56,57} Both T and B lymphocytes have been isolated from cutaneous

basal and squamous cell carcinomas, in numbers comparable to those obtained from biopsies of a delayed hypersensitivity reaction. However, in contrast to the results of Dellen et al.,⁵⁵ no reduction in the number of circulating T lymphocytes in patients with skin cancer, relative to control subjects, could be detected in these studies.^{56,57}

Both of these types of studies can be interpreted as support for the concept that immunologic processes are important in the development and pathogenesis of skin cancer. However, other explanations are equally plausible. For example, the reduced number of circulating T lymphocytes in skin cancer patients could be a consequence of the presence of a large tumor, rather than a causal factor in tumor growth and progression. Similarly, the cellular infiltrate in cutaneous tumors could be related to ulceration or microbial contaminants in the lesion, rather than representative of an immune response against the tumor itself. The specificity of the cellular infiltrates for the autologous tumor must be demonstrated before these observations can be viewed as evidence of immunologic reactivity in human skin cancer. Thus far, the specificity of the antibodies produced by B lymphocytes in the region of these tumors has not been determined, nor has it been established that the infiltrating T lymphocytes are specifically reactive against the tumor tissue.

One study has attempted to assess the systemic immunologic reactivity of nine patients with squamous carcinoma of the skin against autologous tumor tissue.⁵⁸ Although the immunologic tests were not quantitative and the specificity of the reactions was poorly documented, there was an indication of specific immunologic reactivity of serum and of peripheral blood lymphocytes with autologous tumor cells in several patients. This study suggests that some human skin tumors indeed may be antigenic, but the significance of these immunologic reactions in the pathogenesis of the disease remains obscure.

Immunoergic reactions can be used to destroy human skin cancers in certain instances.^{59,60} The induction of contact hypersensitivity reactions at the site of basal cell carcinomas has been shown to cause the regression of these tumors. Whether there is any specific immunologic reactivity directed against tumor antigens, or whether tissue destruction occurs as a nonspecific consequence of the immune reaction against the contact allergen, is not clear.

Immunologic factors may play a role in photocarcinogenesis associated with xeroderma pigmentosum. Patients with this genetic disease have a high incidence of sunlight-induced skin cancers and exhibit an inability to repair UV-radiation-induced lesions in DNA. In such patients, the cells of the lymphoid system that reside in the skin or circulate through it may also accumulate UV-radiation-induced damage in DNA that could lead to immunologic alterations. Such a perturbation in immune function at the site of tumor development may contribute to the pathogenesis of the disease. Although this is purely conjecture, there are several suggestions in the literature of impaired immunologic function

in patients with xeroderma pigmentosum,⁶¹⁻⁶³ which makes it an intriguing possibility.

8.5. Summary

In summary, there are suggestions that at least some human skin tumors may be antigenic and that immunosuppression may lead to an elevated risk of skin cancer development. However, there is little definitive evidence for the involvement of specific immunologic mechanisms in the pathogenesis of cutaneous tumors. The most convincing argument in favor of a contributory role of immunologic factors in skin cancer development is the association between immunosuppressive therapy and an increased risk of skin cancer on the parts of the body exposed to the sun, but this by no means constitutes proof of such an association. The greatest obstacle to translating the immunologic findings from the mouse model to human photocarcinogenesis is the lack, both in man and in the mouse, of an *in vitro* immunologic assay that accurately reflects tumor immunity *in vivo*. Although it is possible to detect and measure the cytotoxic activity of lymphocytes for tumor cells *in vitro* in the mouse system (reviewed in Chapter 10), attempts to detect the activity of suppressor cells by this method have been unsuccessful.^{16,25} An appropriate *in vitro* assay for both the regulatory cells and the effector cells for tumor immunity must be devised before the situation in human subjects can be evaluated.

8.6. References

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Chapter 9

Effects of UV Radiation on Immune Responses in Animals

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9.1. Introduction

There are several immune responses in which the skin serves at the route of entry for the foreign material (antigen) or as the site at which an immunologic reaction occurs. These immune responses, which include delayed-type (lymphocyte-mediated) hypersensitivity, contact hypersensitivity (CHS) (also called allergic contact dermatitis), skin graft rejection, and some forms of immediate (antibody-mediated) hypersensitivity, historically have held a prominent place in dermatologic research. Since exposure to sunlight has been noted clinically both to exacerbate and ameliorate certain cutaneous reactions, it is not surprising that experimental studies were initiated to examine the effects of UV radiation on the elicitation of immune reactions in the skin.

The recent identification of epidermal Langerhans cells as members of the reticuloendothelial system has redirected attention toward the immunologic function of these cells. Questions of current interest concern the role that these cells play in the initiation and/or the elicitation of cutaneous immune responses and the effects of UV radiation on their morphology and function. In addition, the widespread use of UV radiation in the treatment of human skin diseases and the discovery that UV radiation systemically alters the rejection of transplanted UV-radiation-induced tumors in mice recently have prompted investigations of the effects of whole-body exposure to UV radiation on a few systemic immunologic reactions. Thus, for historical reasons, the effects of UV radiation on certain immunologic cells and immune responses are well-characterized and have been investigated extensively, whereas other immune responses have been ex-

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amined only superficially, or not at all in this context. Of necessity, the unequal emphasis of the research is reflected in the unequal emphasis that is given to various immune responses in this chapter. Since the effects of UV radiation on the elicitation and induction of CHS have been studied most extensively, this immune reaction is discussed in considerable detail.

9.2. Mechanisms of Contact Hypersensitivity

9.2.1. Background

9.2.1.1. Description of the Reaction

To analyze the effects of UV radiation on CHS, it is necessary to review what is known about some of the immunologic responses that are initiated or expressed in the skin. Unfortunately, the information available on this topic comes from various experimental systems, and it is typical of these systems that subtle changes in the experimental protocol can produce dramatic differences in the results. The terminology associated with this aspect of immunology is equally varied. Thus, in the literature, CHS also is referred to as *contact sensitization*, *allergic contact dermatitis*, *allergic contact sensitivity*, and various combinations of these terms. The terms *allergic* and *dermatitis* generally are used to describe the reaction in humans, whereas the term CHS usually in conjunction with experimental models in animals. The reaction itself is conveniently subdivided into two parts: the afferent limb, in which the response is first initiated, and the efferent limb, in which the state of sensitivity is expressed. Many synonyms are used to describe the two phases of this process, and the most common ones are listed in Table 9.1.

Contact hypersensitivity is a special form of delayed-type hypersensitivity, in which the antigen is first encountered through the surface of the skin. This type of hypersensitivity is mediated by T lymphocytes, rather than antibody,

Table 9.1
Terms Frequently Employed for Afferent and Efferent Limbs of the Immune Response

Afferent limb	Efferent limb
Induction	Expression
Sensitization	Elicitation
Initiation	Reaction
Priming	
Immunization	

and, as the name implies, the reaction only becomes apparent many hours after the eliciting dose of the antigen has been applied. In CHS, the sensitizing agent consists of a hapten, or small reactive molecule that is conjugated to host cells or proteins (carriers) to form a complete antigen. These antigens, in association with accessory antigen-presenting cells, stimulate the proliferation of a specific subpopulation of T lymphocytes. This clonal expansion of specifically sensitized lymphocytes provides a population of long-lived circulating T lymphocytes that can recognize the original complete antigen when it is encountered a second time. The second encounter with the sensitizing antigen elicits a local inflammatory reaction at the site of antigen contact, which reaches maximal intensity 24 to 48 hr later. Histologically, this reaction is characterized by an intense cellular infiltrate, consisting mainly of T lymphocytes and macrophages.

9.2.1.2. The Reaction in Human Skin

Allergic contact dermatitis is the clinical term used to describe the expression of CHS in human skin. There is an extensive literature characterizing this phenomenon as the manifestation of an immune response that is mediated by lymphoid cells. This reaction is interesting because it results in a high frequency of morbidity. Because CHS in experimental animals seems to be an excellent model for the human disorder, studies of its mechanisms have been of considerable importance for this aspect of dermatology.

The prototype of allergic contact dermatitis in man is poison ivy, or Rhus dermatitis. This reaction has three of the classical features that define an immune response: It is acquired, it is specific, and it has memory. For obvious reasons, it is not possible to test the fourth characteristic which is the ability to be transferred with lymphoid cells. Rhus dermatitis is a good prototype reaction because most individuals who are exposed to the antigen will become sensitized and because the plants responsible for the disorder are ubiquitous. Because there is seasonal variation in the availability of the antigen and a requirement for outdoor activity, this ensures that sensitized individuals will experience clearly defined episodes of the disease. Furthermore, this reaction is elicited through unbroken skin, thus eliminating the complicating factor of an altered skin barrier. This factor is often a component of allergic contact dermatitis to some compounds encountered in industrial settings.

The expression of Rhus dermatitis is characterized by itching, red skin with superimposed blisters. Such pruritic lesions satisfy the classical definition of inflammation by including heat, swelling, redness, pain, and loss of function. Individual lesions conform to the pattern in which the plant was encountered by the skin, indicating that allergic contact dermatitis is expressed only at the sites of hapten application.

9.2.1.3. Inducing Agents

Materials that produce CHS have certain common features. Most have a molecular weight of less than 400 daltons and are lipid soluble, which facilitates passage through skin barriers, particularly the stratum corneum. They are reactive compounds that are able to bind covalently to larger molecules, primarily proteins, and they bind to both soluble and cell surface proteins. The interchangeable terms, *derivatization*, *haptenation*, and *conjugation* all are used to describe the process by which the small reactive haptens bind to these normally nonimmunogenic carriers, converting them to immunogenic conjugates. Thus, the hypersensitivity response is directed toward the entire protein-hapten conjugate, and involves the destruction of some host tissues. The probability that a given hapten will be of clinical importance usually reflects its availability within the human environment. Dinitrochlorobenzene (DNCB) will sensitize most individuals exposed to it, but it is of little clinical importance, since it is rarely encountered even in industrial processes. This high frequency of reactivity to DNCB has led to its widespread use as a member of the integrity of T-lymphocyte-mediated immune reactions in patients with suspected immunologic defects.¹ In contrast, formaldehyde sensitizes only a small fraction of those exposed, but its ubiquitous presence in clothing, in certain plastic resins, and in cleaning agents makes it a significant environmental hazard. Rhus antigen is unique among these three sensitizing agents because it is ubiquitous, and it sensitizes a high proportion of those exposed; thus, the resulting morbidity constitutes a significant public health problem.² Metal salts are also common inducers of this reaction in humans. In animal models, DNCB, trinitrochlorobenzene (TNCB), dinitrofluorobenzene (DNFB) and oxazolone are the sensitizing agents that have been employed most often.

9.2.2. Contact Hypersensitivity as a Cell-Mediated Immune Response

9.2.2.1. Early Studies

The earliest investigations relevant to CHS were concerned with inhibiting delayed-type hypersensitivity reactions, and considerable effort was directed toward this goal. These attempts were made during the period in which neoarsphenamine was the treatment of choice for syphilis, and they were prompted by the high frequency of hypersensitivity reactions that followed its intramuscular injection. In an attempt to prevent this complication, Frei,³ and Sulzberger,⁴ working with humans and guinea pigs, respectively, observed that if an intravenous infusion of neoarsphenamine were given first, the frequency of hypersensitivity reactions was reduced significantly. Although they were unable to produce desensitization of already sensitized subjects, both investigators

recognized correctly that variations in the route of hapten administration produced divergent responses. This work introduced the important concept that the route of administration of the sensitizing agent dictates both the quality and the quantity of the resulting immune response.

Inquiry into the pathogenesis of CHS was stimulated greatly by the report in 1942 that Landsteiner and Chase⁵ had succeeded in transferring this reaction by injecting unsensitized guinea pigs with peritoneal exudate cells taken from sensitized animals. This established for the first time that CHS could be transferred with lymphoid cells, and it contrasted with the failure of Shelmire⁶ to transfer the reaction in humans with injections of serum. This observation was followed by a series of papers by Landsteiner, Chase, and others analyzing the immunologic characteristics of CHS in outbred guinea pigs. Our understanding of the cellular mechanisms of CHS largely results from these pioneering observations.

Continuing the search for ways to inhibit CHS, Chase⁷ explored oral ingestion of the hapten. Although he was unable to decrease the level of sensitivity by feeding DNCB after immunization had occurred, he discovered that feeding the hapten before sensitization resulted in unresponsiveness to a subsequent topical application of the same hapten. This finding emphasized again that the route of first contact with the hapten could determine whether hypersensitivity or specific immunologic tolerance would result from the encounter.

An important observation on the mechanism by which animals acquire hypersensitivity was made by Frey and Wenk in 1959.⁸ They found that the destruction of afferent lymphatics draining the cutaneous site of hapten application prevented sensitization, demonstrating that the induction of CHS requires intact lymphatic drainage to the regional lymph nodes. Continuing along this line, Macher and Chase⁹ carried out an inventive series of studies investigating the effect of excising the site of hapten injection at different times after immunization. In these experiments, they took advantage of the ability to induce CHS in guinea pigs with a single injection of TNBC in one ear. The temporal requirements for sensitization were determined by excising injected ears sequentially from different groups of animals. When ears immunized with TNBC were excised 12 hr later, only 1 of 28 guinea pigs was sensitized (4%). In contrast, 16 of 20 animals were sensitized when their ears were left undisturbed (80%). Excision of the TNBC treated ears at 24 or 48 hr after sensitization in two additional groups of animals produced an intermediate proportion of sensitized animals (14% and 61%, respectively). One interpretation of this observation is that CHS is acquired relatively slowly, and maximal sensitization requires the persistence of the hapten at the site of immunization for 48 hr. A second and more plausible interpretation arose from their next set of experiments, which dealt with those animals in which sensitization failed to occur. Animals not sensitized in the first study because of the premature excision of

the site of hapten administration were then exposed to a normally immunizing regimen in which TNCB was applied to the remaining ear. A significant percentage of the animals remained unresponsive after the second application of hapten, indicating that they had developed immunologic tolerance during the first procedure. Additional studies demonstrated that a significant amount of injected TNCB leaves the site of application within minutes and reaches the systemic circulation via venous outflow. Furthermore, the incidence of tolerant animals among those subjected to early ear excision was similar to that observed when TNCB was injected in similar amounts either intradermally or intravenously prior to the usual immunization protocol. From these studies, Macher and Chase drew three conclusions: (1) The hapten that remains in the ear after early outflow constitutes the sensitizing depot. (2) The fraction of allergen that escapes from the sensitization site soon after injection induces a state of tolerance in most animals from which the sensitizing depot has been removed. A similar state of tolerance can be induced by injecting the sensitizer systemically in an amount equivalent to that which escapes during normal sensitization. (3) The eventual degree of reactivity exhibited by the animals is therefore the composite result of two independent immunologic processes: The tolerogenic effect of escaped material, and the sensitizing effect of localized material.⁹

Studies that have assessed the balance between hypersensitivity and suppression also have been conducted in humans. Insight into these opposing effects may be gained from a series of papers by Lowney, in which methods of inducing tolerance were explored. In the first study,¹⁰ human subjects were sensitized through normal skin by the repeated application of small amounts of DNCB. Subjects treated in this manner developed lower reactivities to DNCB than did control subjects sensitized at one time with large amounts. Furthermore, among several protocols, those subjects who could never be sensitized by topical application of DNCB came preferentially from protocols that utilized repeated exposures to small amounts of DNCB. Apparently, each type of sensitization elicited two responses, one reactive and one suppressive, and the balance between them was determined by the particular regimen employed.

In Lowney's second study, the capacity of a particular route of hapten administration to induce both hypersensitivity and suppression simultaneously was even more clearly demonstrated.¹¹ One cohort of 17 subjects received their first exposure to DNCB through the buccal mucosa. Interestingly, eight of these subjects never developed hypersensitivity to DNCB, demonstrating that the buccal mucosa route was more likely to induce suppression than the cutaneous route. The seven who were sensitized in this protocol were subsequently exposed to a "normal" percutaneous regimen of sensitization, as were the appropriate control subjects who received two normal sensitizing regimens. Those who had first developed CHS via buccal mucosa remained significantly less responsive

after this second course of sensitization, indicating these subjects had acquired simultaneously both hypersensitivity and some measure of unresponsiveness.

The nature of the complete antigens that induce hypersensitivity or tolerance is not known. Whether these are hapten-derivatized cell membranes, proteins, viable cells, or some combination of these cannot be inferred from the experiments described above. Perhaps each of these antigens triggers a different immunologic pathway. However, this possibility is purely speculative, and additional studies are required to clarify the issue.

9.2.2.2. Studies in Inbred Animals

Recent studies on CHS have been aided greatly by the availability of inbred strains of laboratory rodents. Because of the genetic homogeneity that results from inbreeding, reactions to procedures such as immunization and elicitation are more uniform. Also, it is possible to transfer lymphoid cells among genetically identical (syngeneic) animals without risking immunologic injury of the recipient animal by the transfused cells (graft versus host reaction) or immunologic destruction of the transfused cells by the recipient (host versus graft reaction). Furthermore, it has been shown that certain immune reactions involve collaboration among different types of cells, some of which must have genetic identity at particular chromosomal loci in order for an effective interaction to occur. The use of syngeneic animals ensures that such genetically restricted immune reactions can occur with adoptively transferred cells.

The usefulness of mice for studying CHS was at first impeded by the failure of immunized animals to develop visible skin reactions in response to epicutaneously applied haptens. This problem was overcome, however, by the observation of Asherson et al.¹² that the amount of cutaneous edema elicited by application of the reactive haptens on the ear surface correlated with sensitization and could be measured with a spring-loaded engineer's micrometer. Subsequently, Miller and Vadas and their associates employed a second successful assay in which the emigration of radiolabeled lymphoid cells into the ears, the site of elicitation, was measured by excising the ears and determining the amount of radioactivity present.^{13,14} Because the development of these two assays, mice and hamsters now are used for studies of CHS.

With such models, insight has been gained into the balance between specific unresponsiveness and hypersensitivity by using methods that favor one reaction over the other. Working from the observation that delivering reactive haptens through a stomach tube could produce specific immunologic unresponsiveness, Polak et al.¹⁵ demonstrated that this unresponsiveness was prevented by pre-treatment of mice with cyclophosphamide, an inhibitor of cell division. This suggested that the suppression observed after oral ingestion of a hapten is an active process that requires cell division. Subsequently, Asherson et al.¹² demon-

strated that the administration of either oxazolone or TNCB resulted in the generation of at least two different generic populations of suppressor cells, one a B cell population, and the other a T-cell population. These observations underscore the complexity of the cellular events resulting from an immunization procedure. More recently, Greene, Dorf, Benacerraf, and their collaborators,¹⁶ as well as Miller, Claman, Moorhead, and associates,^{16a} have provided evidence for as many as three distinct subpopulations of T lymphocytes that participate in the regulation of CHS responses in mice. In sum, the interpretations of *in vivo* immune reactions are complicated by the realization that these responses are composites of a family of responses, some of which are antagonistic and some of which are synergistic. Furthermore, these responses may differ in their times of onset and peak reactivity.

9.2.3. Role of Antigen-Presenting Cells in Contact Hypersensitivity

9.2.3.1. Accessory and Dendritic Cells

The development of CHS after exposure to a contact sensitizing agent is dependent upon cellular collaboration among lymphoid cells. In the induction of CHS, as in numerous other immune responses, antigens must be presented to T lymphocytes by an “accessory cell,” usually of the macrophage lineage. This collaborative process is genetically restricted, in that optimal sensitization will occur only when the lymphocyte and the antigen-presenting cell are genetically identical in certain regions of the major histocompatibility complex. Likewise, under certain circumstances, the elicitation of an immune response may require both antigen presentation and genetic restriction, although for CHS reactions *in vivo* such requirements have not been demonstrated.

Recent evidence indicates that an antigen-presenting accessory cell is also required for lymphocyte activation and proliferation to occur.¹⁷ The elegant work of Rosenthal, Shevach, Green, and collaborators during the mid- and late 1970s firmly established that macrophages, usually identifiable by their capacity to adhere to plastic surfaces and to phagocytose particulate material, are essential in the process of antigen recognition by immunocompetent lymphocytes.¹⁸ The precise form of the immunogenic moiety presented by the macrophage to the lymphocyte has yet to be elucidated. However, it involves the participation of cell surface molecules on the macrophage, which are encoded by genes within the major histocompatibility complex.

As the evidence began to accumulate in favor of the macrophage as the main antigen-presenting cell, another adherent, but nonphagocytic, cell was reported to carry out the function of antigen presentation. Work by Steinman and his associates¹⁹ documented that there is a minor subpopulation of cells in the spleen, termed dendritic cells, that is extraordinarily efficient in presenting

antigen. These cells comprise about 1% of the spleen cells; they bear gene products of the major histocompatibility complex on their surfaces, and their antigen-presenting function exhibits genetic restriction. They are also unusual in that they lack the identifiable surface markers of differentiated B cells (surface immunoglobulin), T cells (Thy-1 antigen) and of macrophages (Fc receptors). Functionally, dendritic cells stimulate allogeneic²⁰ and syngeneic²¹ mixed leukocyte reactions at exceedingly low densities. They also are able to present antigen for a secondary immune response *in vitro*. Most significant in the context of contact hypersensitivity is the recent observation that dendritic cells present antigen *in vitro* for the induction of cytotoxic T lymphocytes directed against haptenated cells.²²

9.2.3.2. Langerhans Cells

At the same time that the work on dendritic spleen cells was being carried out, immunologists and dermatologists began to reexamine the Langerhans cell with the view that this dendritic epidermal cell also might carry out immunologic functions. The epidermis is a multilayered tissue composed mainly of keratinocytes, which provides a protective barrier between an animal and its environment. Differentiated keratinocytes arise from a proliferating subpopulation of epidermal cells, and after a series of maturational steps, these cells flatten into hexagonal leaflets that are bound together, forming a semipermeable surface coat. Approximately 95% of the cells in the epidermis are keratinocytes; thus, the processes of cell proliferation and barrier formation represent the primary function of the epidermis. However, about 3% of the cells comprising the epidermis are Langerhans cells, which are distributed suprabasally throughout the epidermis in a regular, gridlike network. Each Langerhans cell possesses a central body with a lobulated nucleus and several long dendritic processes extending outward from the central cell body and passing between the keratinocytes. Morphologically, these cells resemble melanocytes, the third main cellular component of the epidermis, and for many years, the Langerhans cells were thought to represent premelanocytes. However, it is possible to distinguish between these two types of cells by histochemical, immunochemical, and ultrastructural techniques.

The hypothesis that Langerhans cells might play a role in immunologic processes was put forward first by Silberberg.^{23,24} The evidence brought forth to support this hypothesis was primarily circumstantial, however, and relied mainly on morphological studies of tissue sections. Silberberg and her co-workers demonstrated in a series of electron microscopic analyses that lymphocytes come in direct contact with epidermal Langerhans cells at sites of contact hypersensitivity reactions²³ and that Langerhans cells can be found in dermal lymphatic vessels and draining lymph nodes under certain conditions.²⁵ Wolff and

Schreiner concluded that Langerhans cells also can take up and process protein antigens in the skin.²⁶ Additional support for an immunologic role of Langerhans cells was provided by the morphologic studies of Shelley and Juhlin that showed that contact-sensitizing haptens accumulated preferentially within Langerhans cells in the epidermis.²⁷

A major advance occurred in 1977 with simultaneous reports from three laboratories that Langerhans cells possessed determinants on their surface membranes that identified them as belonging to the macrophage-monocyte lineage.²⁸⁻³⁰ These surface determinants, Fc receptors which bind immunoglobulins, C3b receptors which bind a component of complement, and Ia molecules (or B-cell alloantigens in human skin³¹) which participate in antigen presentation, typically are found on immunologically active cells. It is quite clear that murine Langerhans cells express Ia determinants on their surface and that this property is shared by antigen-presenting macrophages. What is not clear is whether these determinants are found on epidermal cells other than Langerhans cells. When fluorescent antibodies against Ia determinants are used to label Ia-bearing cells in the epidermis, only about 5% of the epidermal cells are positive.^{29,30,32,33} However, when cytotoxicity tests are performed using anti-Ia antibody and complement, as many as 90% of epidermal cells are Ia positive.³⁴⁻³⁶ Interpretation of these findings is complicated further by the observation that during some autoimmune or inflammatory reactions, Ia antigens appear to be expressed on nearly all epidermal cells.³⁷ Nonetheless, there is no doubt that the Langerhans cell has all the attributes of an antigen-presenting cell. In fact, recent studies with radiation chimeras have shown that these cells are derived from precursors in the bone marrow, and thus are not epithelial in origin.^{38,39}

Work by Streilein, Bergstresser, and co-workers^{40,42-44} suggests that Langerhans cells play an important role in the induction of CHS. These investigators correlated the density of Langerhans cells in various sites with the ability of these sites to support the induction of CHS. In mice, the density of Langerhans cells in tail skin is significantly lower than in body wall skin. For example, in mice of the C57BL/6 strain the density of Langerhans cells in tail skin is only about 20% of that found in body wall skin. When these mice were exposed to a normally sensitizing regimen of DNFB through the tail, they failed to develop CHS. Moreover, a subsequent application of DNFB to abdominal skin also failed to induce CHS, whereas other haptens readily induced CHS in these animals.⁴⁰ Thus, exposure of the mice to a sensitizing agent through skin that is deficient in Langerhans cells resulted in the induction of specific immunologic unresponsiveness, instead of CHS. More recently, this unresponsiveness was shown to be associated with antigen-specific suppressor T lymphocytes.⁴¹

Similar results were obtained from experiments on Syrian hamsters, which also have been shown recently to serve as excellent models for studies of CHS.⁴²

These animals possess a cheek pouch that is considered to be an immunologically privileged site because foreign tissues placed within it survive considerably longer than do comparable grafts placed at other sites. This epithelial pouch lacks a lymphatic drainage pathway to the regional lymph node and in addition contains relatively few Langerhans cells.⁴³ Streilein and Bergstresser⁴⁴ observed that painting intact cheek pouch epithelium with a contact sensitizing agent resulted in the induction of specific immunologic unresponsiveness. Because this result could have been due to oral ingestion of the hapten, rather than entry through cheek pouch epithelium, this tissue was grafted to the body wall, and sensitization was attempted through this heterotopically placed graft. Again, immunologic tolerance was induced, and furthermore, the unresponsiveness could be transferred to normal animals by injecting them with lymphoid cells from the tolerant hamsters. Thus, active suppression can result from the interaction of hapten with skin that is depleted of Langerhans cells. This implies that Langerhans cells play an important role in the induction of contact hypersensitivity.

More direct demonstrations of the immunologic functions of Langerhans cells have come about through the use of these cells as antigen-presenting cells for *in vitro* reactions and for the induction of contact hypersensitivity *in vivo*. Langerhans cells can be separated physically from keratinocytes in disaggregated guinea pig epidermis by making use of their surface Fc receptors to bind antibody-coated erythrocytes. These large rosettes, composed of a Langerhans cell surrounded by erythrocytes, can be separated from the keratinocytes by sedimentation. Langerhans cells isolated in this manner are capable of presenting antigens *in vitro*, as determined by their ability to induce antigen-specific proliferation of sensitized T lymphocytes.⁴⁵ In the mouse, this type of study has been hampered by an inability to obtain sufficiently pure Langerhans cell preparations using approaches similar to those that have been successful in the guinea pig. Nonetheless, crude epidermal cell suspensions have been shown to present antigens in this *in vitro* immunologic assay.⁴⁶ The activity of these cells is abrogated by treating the Langerhans cell suspension with anti-Ia antiserum and complement, which eliminates cells expressing Ia surface determinants. Furthermore, the ability of these cells to induce lymphocyte proliferation is subject to the same genetic restrictions as those described earlier for macrophages, thus providing an additional link between the functional capabilities of these cells.^{47,48}

Epidermal cell suspensions also have been compared with different macrophage-containing populations for their ability to present antigen for *in vivo* immunization. Contact hypersensitivity can be induced in mice by a subcutaneous injection of macrophages that have been derivatized with a hapten *in vitro*.⁴⁹ Using this approach, Ptak et al.⁵⁰ and Tamaki et al.⁵¹ have compared the ability of trinitrophenol (TNP)-derivatized epidermal cells and splenic or peritoneal macrophages to induce CHS after administration by various routes. In these

studies, CHS was assessed by measuring ear swelling 24 hr after applying TNCB to the ear surfaces. Both groups found that CHS could be induced with TNP-conjugated adherent spleen cells or epidermal cells injected subcutaneously, but the reaction was greater and persisted longer after immunization with the epidermal cells. Interestingly, intraperitoneal injection of the derivatized cells resulted in the induction of CHS with epidermal cells but resulted in the induction of specific unresponsiveness with adherent spleen cells. The results with intravenous injection of the two cell populations is not yet clear. Ptak et al.⁵⁰ reported that TNP-conjugated epidermal cells were the only population that could induce CHS after intravenous injection. Splenic and peritoneal macrophages were ineffective when administered by this route. On the other hand, Tamaki et al.⁵¹ found that neither epidermal cells nor splenic macrophages could induce CHS by this route, and that such immunizations induced specific unresponsiveness. Additional experiments are required to resolve this discrepancy. However, these studies clearly show that the Langerhans cell in the epidermal cell preparations are capable of serving as antigen-presenting cells for the induction of CHS *in vivo*.

9.3. Effects of UV Radiation on the Contact Hypersensitivity Reaction

9.3.1. Elicitation of Contact Hypersensitivity in UV-Irradiated Skin

The possibility that UV radiation might alter the expression of CHS was first introduced by Haniszko and Suskind.⁵² They employed a guinea pig model in which erythema and edema at the site of application of the test compound indicated positive reactions. The sites of elicitation were irradiated with suberythemal doses of UV radiation from a radiation source that emits a peak level of radiation between 290 and 310 nm. They observed that a radiation schedule of 12 exposures delivered over 14 days diminished significantly the reaction elicited by DNB in the irradiated sites compared with those produced in unirradiated contralateral body wall skin. This diminution persisted for as long as 2 wk after discontinuing the irradiation, and it could be observed for up to 4 wk after sensitization. However, all animals exhibited positive responses in irradiated sites at the highest eliciting dose of DNB, indicating that the elicitation reactions had been reduced, but not completely prevented. At the doses of UV radiation used in this study, systemic suppression was not noted in the irradiated animals.

This phenomenon was reinvestigated more recently by Morison et al.,⁵³ who assessed the impact of UVB radiation or psoralen plus UVA radiation (PUVA) on CHS to DNB in Hartley-strain, albino guinea pigs. Radiation exposures were given daily, beginning on the day of sensitization and continuing until the

time of challenge and were administered at a site adjacent to that used for sensitization. Their data demonstrate that relatively high doses of UVB radiation or PUVA, sufficient to cause erythema and edema could partially inhibit the expression of CHS. This study leaves some questions unanswered, however, because the diminution in the reaction in the animals treated with PUVA occurred only when they were treated with PUVA beginning at the time of sensitization. The same PUVA treatment beginning 14 days after sensitization did not affect the expression of CHS in the site exposed to UVA radiation. These experiments suggest the possibility that, during the development of CHS, there is a period during which PUVA treatment can interfere with the induction of the reaction, implying that PUVA treatment may have a systemic effect. Meyer et al.⁵⁴ also reported that CHS to DNCB in guinea pigs is suppressed by three erythemagenic treatments with PUVA given close to the time of induction. However, since both the sites of elicitation and induction were included in the area exposed to UVA radiation, it is not clear whether PUVA was affecting the sensitization or the elicitation phases of the reaction, or both.

9.3.2. Induction of Contact Hypersensitivity through UV-Irradiated Skin

In retrospect, the work of Haniszko and Suskind⁵² demonstrating a local effect of UVB radiation on the elicitation of CHS suggests that UVB radiation also may exert a local effect on the induction of CHS. However, it was not until after the resurgence of interest in Langerhans cells, and more particularly, in their putative immunologic functions that this possibility was first tested. Toews, Bergstresser, and Streilein,⁴⁰ working with C57BL/6 mice, assessed the impact of very low doses of UVB radiation on the integrity of Langerhans cells, as judged by cell membrane ATPase activity, and on the induction of CHS to DNFB. Ultraviolet radiation was administered by unfiltered FS20 sunlamp fluorescent tubes, which emit a continuous ultraviolet spectrum with a peak at 313 nm and significant output in the sunburn or erythema spectrum. The mice were irradiated once a day for 4 days with 100 J/m² of UV radiation on a shaved, 2.5-cm² area of abdominal wall skin, and then the skin was painted with a standard immunizing regimen of DNFB. This radiation schedule produced modest changes in the visual and histopathologic appearance of the irradiated skin, including some epidermal thickening, but no significant cellular infiltrate. The changes that were seen were confined to the epidermis.

When the usual immunizing procedure was conducted on abdominal wall skin pretreated in this way with UV radiation, normal immunization did not occur, as measured by swelling of ears that were not exposed to UV radiation. Moreover, there was a direct correlation between the number of ATPase-staining Langerhans cells in the skin and the capacity of such skin to permit sensitization during the period of time after UV radiation treatment when Langer-

hans cells were returning to the irradiated skin. In a subsequent experiment, mice that had received their first exposure to DNFB through UV-radiation-treated skin, then were given a normally immunizing regimen through untreated dorsal body wall skin. These animals were unresponsive to the attempted conventional immunization and the unresponsiveness was specific for DNFB. Most importantly, however, this capacity to induce specific unresponsiveness through UVB-treated skin was local, in that it was limited to the site of UV radiation exposure. Irradiation of abdominal wall skin did not alter the capacity of dorsal skin to produce CHS. This result also demonstrates that the elicitation phase of the CHS reaction is unaffected by low doses of UV at a distant site. It was suggested that this local effect of UV radiation was related to the morphologic and enzymatic perturbations observed in the Langerhans cells.

These observations were confirmed by Lynch et al.,⁵⁵ although they employed a dose of UV radiation that was considerably higher than that used by Toews et al.⁴⁰ In this study, the abdominal wall skin of C3H mice was irradiated daily with sunlamps for 6 days. Painting a hapten on the irradiated area resulted not in hypersensitivity, but in unresponsiveness, as shown by subsequent attempts at sensitization through an unirradiated site. Application of the sensitizing agent on unirradiated skin in a similar cohort of animals resulted in the development of CHS, indicating that systemic unresponsiveness had not been produced by this regimen of UV radiation.

9.3.3. Systemic Suppression of Contact Hypersensitivity by UV Radiation

Pretreatment of mice with much higher doses of UV radiation also has been shown to prevent the development of CHS to antigens that are applied subsequently to unirradiated skin.⁵⁶ This observation was made originally in studies of the systemic immunologic capabilities of mice during carcinogenesis by UV radiation; hence, the radiation doses were derived from protocols that led to skin cancer induction. This is in contrast to the much lower doses of UV radiation used to inhibit sensitization on UV-irradiated skin, in which case the doses are based on those required to produce morphologic alterations in Langerhans cells.⁴⁰

The first studies of this phenomenon were carried out by Kripke, Jessup, and colleagues.^{57,58} They sensitized mice by subcutaneously injecting DNFB in dimethylsulfoxide, an inflammatory agent, and then measured the response in the footpad to injected DNCB in dimethylsulfoxide. It is not clear whether this system is more akin to CHS or to classical delayed-typed hypersensitivity in terms of the nature of the antigen; however, many of the findings with this system have been reproduced using more conventional forms of contact sensitization.^{59,60} In the studies of Jessup et al.,⁵⁷ the shaved dorsal surface of mice was exposed to approximately 7 kJ/m² of UV radiation from FS40 sunlamps,

3 times per week for various periods of time. After cessation of the irradiation, the mice were sensitized on the abdomen by subcutaneous injection of DNCB, and the reaction was elicited in the footpad 1 wk later. Mice that were exposed to UV radiation had a decreased response to the challenge dose of DNCB. The cellular basis for this depressed reactivity to DNCB was analyzed in a series of cell transfer experiments.⁵⁷ Transfer of immune lymphocytes into UV-irradiated animals demonstrated that the effector portion of the immune response to DNCB, measured by footpad swelling, was intact in UV-irradiated mice. Furthermore, lymphocytes from UV-irradiated mice were able to respond normally to DNCB, provided that they were removed from the UV-irradiated host and used to repopulate lethally X-irradiated animals. These results implied that the systemic alteration in UV-irradiated mice that prevented DNCB sensitization occurred at a very early step in the immune response, most likely at a level of the macrophages or Langerhans cells that are involved in the uptake, processing, and presentation of antigen to the lymphocytes. One interesting feature of this UV-radiation-induced suppression is that it is transient in nature. After 2 or 3 months of chronic irradiation, these animals regain their ability to be sensitized, even in the face of continued irradiation.

Subsequent studies by Noonan et al. have examined this phenomenon in greater detail, using the more conventional contact hypersensitivity reaction to TNBC or DNFB in mice.^{56,60,61} They found that a single dose of UV radiation (approximately 20 kJ/m² from FS40 sunlamps) was sufficient to prevent CHS in mice that were subsequently sensitized and challenged at unirradiated sites. The amount of reactivity induced by the sensitizing agent was inversely proportional to the log₁₀ dose of radiation. In addition, more than 24 hr had to elapse between the time of a single UV radiation exposure and the time the sensitizing agent was applied in order for suppression to occur, and this unresponsive state persisted for approximately 2 to 3 wk. The unresponsiveness was associated with the appearance of antigen-specific suppressor T lymphocytes, which, upon transfer to a syngeneic animal, prevented the induction of CHS to the same antigen.

The wavelength dependence of this suppression of CHS has been determined recently. Removal of wavelengths below 315 nm by means of a Mylar filter removed the suppressive activity from FS40 sunlamps.⁶⁰ Preliminary reports of studies with a narrow-band monochromator indicate that the most efficient wavelengths for inducing suppression to contact sensitzers are in the 260 to 270 nm range.^{56,61} In combination with psoralen, UVA radiation (320 to 400 nm) also can suppress CHS reactions systemically.⁶² This suggests that the suppressive effect of UV radiation on CHS may originate from its effects on the DNA of particular cells in the skin. However, it is not yet known whether the suppressive effects of PUVA on CHS involve the same immunologic mechanisms as those evoked by UV radiation of shorter wavelengths. Similar results

also have been reported in another study of mice,⁵⁵ along with the somewhat surprising finding that UVA radiation alone was almost as effective as PUVA in producing systemic suppression of CHS. This effect may have been caused by UVB radiation from the unfiltered bulbs, but evaluation of the finding is difficult because no information was given as to the phototoxic response to radiation or PUVA treatment.

9.3.4. Mechanisms

9.3.4.1. Effects of UV Radiation on Langerhans Cells

An obvious question that arises from these studies on the suppression of CHS by UV radiation is whether any of these three immunologic alterations, decreased elicitation in UV-irradiated skin, altered sensitization through UV-irradiated skin, or systemic suppression following sensitization and challenge of unirradiated skin, results from a direct or indirect effect of UV radiation on epidermal Langerhans cells. This possibility is attractive because of the suggestion that Langerhans cells exhibit a high degree of sensitivity to UV radiation. This suggestion arose in 1959 from the studies of Fan et al.⁶³ on guinea pig skin using a gold chloride staining technique and light microscopy. A similar report using human epidermis examined by electron microscopy was published by Zelickson and Mottaz in 1970.⁶⁴ In contrast, Wolff and Winkelmann failed to observe changes in Langerhans cells in guinea pig skin that was irradiated with UVC radiation at doses that increased both the density and tyrosinase activity of melanocytes.⁶⁵ However, this discrepancy has been attributed to a difference in the time period that elapsed between exposure to UV radiation and the examination of the skin.⁶⁶

More recent studies using antibodies directed against Ia antigens on Langerhans cells and histochemical stains for cell membrane ATPase, which is present in high concentration in Langerhans cells, have shown that morphologic, antigenic, and enzymatic changes occur in these cells after exposure of the skin to UV radiation. As mentioned earlier, the work of Toews et al.⁴⁰ demonstrated that UVB radiation delivered in small doses to mouse skin produced significant alterations in Langerhans cells. The density of Langerhans cells, as judged by ATPase staining was reduced from 800 cells/mm² of epidermis to fewer than 50 cells/mm² within 24 hr after irradiation. The Langerhans cells that could be found after irradiation had abnormal morphology; the cells were highly contracted, darkly stained, and lacked dendritic processes. Aberer et al.⁶⁶ confirmed this finding in both mouse and human skin and showed, in addition, that after UV irradiation, Langerhans cells also lose their ability to react with antibody directed against Ia antigens. Since these Ia antigens are recognition structures that are probably essential for optimal antigen presentation and cellular collabo-

ration, this result implies that UV radiation may affect this function of Langerhans cells, in addition to altering their morphology.

Similar morphologic and histochemical alterations of Langerhans cells have been reported after PUVA treatment of mice and guinea pigs. Repeated treatment of mice with topical PUVA was found to decrease the number of Ia-positive cells in the epidermis,⁶⁷ presumably due to an alteration in the surface properties of the Langerhans cells. This effect was dose-dependent and persisted for up to 5 wk after the cessation of treatment. A similar regimen of PUVA treatment also decreased the number of ATPase-staining Langerhans cells in the epidermis of mice,⁵⁵ but this number began to increase again at 8 days after the treatment. In the guinea pig, PUVA treatment, but not psoralen alone or UVA alone, also resulted in a marked decrease in the number of ATPase-positive cells at the site exposed to radiation; there was no alteration in Langerhans cells at contralateral unexposed sites.⁶⁸ The number of ATPase-positive cells reached a minimum between 5 and 7 days after PUVA treatment and returned to the normal number by day 14 after treatment.

One question that remains unresolved from these studies is the fate of the UV-radiation-damaged Langerhans cells. Toews et al.⁴⁰ originally interpreted the loss of ATPase-positive cells as indicating that these cells had been destroyed or had emigrated out of the epidermis. However, the study by Aberer et al.⁶⁶ suggests that although there is a reduction in the number of ATPase-positive cells after UV irradiation of the skin, many of these cells are still present in the epidermis and can be detected by electron microscopy. Additional data from Streilein et al.⁶⁹ demonstrated that UV radiation, delivered at 100 J/m² per day over 4 days did not alter the immunogenicity of skin grafts placed on congenic mice that differed genetically from the graft donors only in the I region of the major histocompatibility complex. Since Langerhans cells are the main source of this antigen in skin, this result implies also that UV radiation in these doses does not destroy the Langerhans cells. Thus, it is not clear whether a fraction of Langerhans cells dies or emigrates from the epidermis in response to an injurious effect of UV radiation, or whether the cells merely lose their staining reactions while remaining in the epidermis. Furthermore, the reappearance of stained cells after irradiation could represent the reappearance of markers on residual cells, the influx of new Langerhans cells into the epidermis from the blood, or an infiltrate of inflammatory cells. In this context, epidermal thickening and increased keratinization is a consequence of exposing the skin to even these low doses of UV radiation. At higher doses, inflammatory cells are present, necrosis occurs, and exudative crusts form over the epidermis. These events clearly alter the transmission of UV radiation through the skin and actually may protect underlying Langerhans cells from additional UV-radiation-induced injury. Thus, protocols examining the effects of repeated exposures of skin to UV radiation are complicated by these dynamic changes. These changes in the

epidermis are an important consideration in interpreting studies carried out at different times after a single exposure to UV radiation and in studying the effects of multiple UV radiation exposures.

As discussed earlier, Toews et al.⁴⁰ attempted to test the functional significance of these UV-radiation-induced changes in Langerhans cells by applying a contact sensitizer to irradiated skin. A correlation was observed between the effectiveness of the sensitizing dose of DNFB and the number of ATPase-positive cells in the epidermis. However, this correlation does not provide direct proof of a causal relationship between these two events. A more direct approach to this problem has been taken by Sauder et al.,⁷⁰ who drew on the capacity of TNP-derivatized epidermal cells to induce CHS to TNCB when injected subcutaneously. Employing FS20 sunlamps, they observed that 198 J/m² of UV radiation, delivered to the cells *in vitro* before derivatization with TNP, prevented optimal sensitization. Moreover, animals receiving such cells were suppressed significantly in their capacity to develop CHS after skin painting 6 days later with a normally sensitizing dose of TNCB. In a subsequent experiment, this suppression was adoptively transferred with spleen cells to untreated recipients, and this transfer could be abrogated by pretreatment of the cell suspension with anti-theta antiserum and complement. These observations suggest strongly that the suppression induced by UV-irradiated epidermal cell preparations is an active process and that it is mediated by theta-antigen-bearing T lymphocytes. In these studies, the amount of suppression of CHS was directly dependent upon the dose of UV radiation administered to the epidermal cells and on the number of UV-irradiated epidermal cells injected. Furthermore, the irradiation had no detectable effect on the epidermal cells, as judged by survival of the entire cell suspension and by the survival of the Ia-positive subfraction of the cells within the suspension (Langerhans cells). This work strongly supports the hypothesis that UV radiation is capable of interrupting directly the antigen-presenting capacity of the Langerhans cells within the epidermis.

Recent work by Stingl et al.⁴⁶ also supports the hypothesis that UV radiation directly affects the antigen-presenting function of Langerhans cells and gives some insight into the nature of the alteration. Rather than studying the ability of haptenated epidermal cells to induce CHS *in vivo*, these investigators took advantage of the capacity of epidermal cell preparations to serve as antigen-presenting cells in an *in vitro* correlate of a delayed-type hypersensitivity response. Although it has not yet been possible to carry out such studies in mice with purified Langerhans cells, there is evidence that these cells are, in fact, required for antigen presentation in this system⁴⁶; however, the possibility that keratinocytes participate in this reaction in some way has not been ruled out. In this *in vitro* reaction, the proliferation of sensitized T lymphocytes in response to the specific antigen is measured by incorporation of tritiated thymidine. T lymphocytes from either BALB/c or C3H/HeN mice, immune to purified protein

derivative of tuberculin and to dinitrophenylated ovalbumen, were cocultured with antigen-pulsed cells from either peritoneal exudates (consisting of about 90% macrophages) or unpurified epidermis (consisting of about 2 to 4% Langerhans cells). Both sources of antigen-pulsed stimulator cells could, in a genetically restricted way, induce a vigorous proliferative response in the immune T lymphocytes. This response could be abolished by pretreating the epidermal cells with a monoclonal anti-Ia antibody and complement without killing significant numbers of these cells. This strongly suggests that Langerhans cells were responsible for antigen presentation by the epidermal cell preparation. When epidermal cells were exposed, either before or after the antigen pulse to UV radiation from Sylvania F20T12 fluorescent bulbs, there was a dose-dependent inhibition of their ability to induce the proliferative response measured after 4 days in culture. Mixing studies demonstrate¹ that this effect was not attributable to the release of toxic epidermal cell products after irradiation but was attributable to a direct effect of UV radiation on the function of the epidermal cell preparation.

These studies clearly demonstrate the capacity of UVB radiation to interfere directly with antigen presentation by epidermal Langerhans cells, and they provide insight into the *in vivo* studies that employed similar doses and wavelengths of UV radiation. Sensitization through a UV-irradiated site probably results in an alteration in the presentation of the antigen by Langerhans cells, and this alteration is probably responsible, in some way, for the resulting suppression. However, the enzymatic, antigenic, and morphologic alterations that can be seen in Langerhans cells after UV irradiation *in vivo* are changes that only correlate with the altered sensitization phase of CHS. There is no direct evidence demonstrating a causal relationship between these visible changes in Langerhans cells and the induction of active suppression. Another point that remains unresolved is whether the keratinocytes play any role in this process. Although it has been demonstrated by deletion experiments that the Langerhans cells are required for antigen presentation *in vitro*, these experiments do not rule out the possibility that the keratinocytes contribute to the resulting proliferation of lymphocytes by supplying chemical mediators or growth factors. This possibility is given some credence by the finding that keratinocytes produce a soluble factor that enhances the proliferation of T lymphocytes.⁷¹ Another question left unanswered is whether the induction of suppression *in vivo* after application of the sensitizer onto a UV-irradiated site is an active or a passive process. Ultraviolet-irradiation of the Langerhans cells may induce changes in these cells that cause them to present antigen in a different way, thus actively leading to suppressor cell induction. Alternatively, this finding can be interpreted based on the conclusions of Macher and Chase.⁹ They showed that bypassing the sensitizing antigen depot in the skin resulted in the induction of specific unresponsiveness and that the eventual degree of contact sensitization achieved was the result

of two relatively independent processes, one suppressive and one reactive. Thus, it is possible that UV radiation inactivates the cutaneous route of sensitization by damaging Langerhans cells, thereby passively permitting the expression of specific suppression induced by direct passage of the hapten into the venous circulation, or perhaps even by oral ingestion of the hapten.

Whether these effects of UV radiation on Langerhans cells are responsible for the decreased elicitation of CHS in irradiated skin is not known. The studies of Toews et al.⁴⁰ and Jessup et al.⁵⁷ show that neither the low nor the high doses of UV radiation that affect the induction of CHS interfere with the elicitation of the reaction in unirradiated skin. However, as yet, no attempts have been made to correlate UV-radiation-induced alterations in Langerhans cells with the reduced reactivity of irradiated skin. Many questions about the role of Langerhans cells in the elicitation of the CHS reaction *in vivo* are unanswered, i.e., whether the requirements for antigen presentation are the same for the induction, the elicitation, and the expression of the CHS response, and what role Langerhans cells play in each of these phases of the reaction.

Finally, these studies do not provide any insights into the systemic suppression of CHS by high doses of UV radiation, in which both sensitization and elicitation is performed on unirradiated skin. Preliminary data from Noonan et al.⁷² suggest that the morphologic alterations induced in Langerhans cells by UV radiation are neither responsible for nor related to the induction of systemic suppression. Using monochromatic UV radiation, these investigators found that 320-nm UV radiation could induce systemic suppression of CHS without producing any detectable alterations in the morphology or ATPase staining of the Langerhans cells. Conversely, very low doses of 270-nm radiation produced marked alterations in the Langerhans cells without inducing systemic suppression of CHS. Thus, it appears that either this systemic effect of UV radiation is not mediated through its effects on Langerhans cells or, alternatively, the Langerhans cells are involved in the initiation of the effect, but the morphological alterations induced in them by UV radiation represent an unrelated event.

9.3.4.2. Effects of UV Radiation on Other Antigen-Presenting Cells

It has been known for some time that exposure of lymphoid cells to sub-lethal doses of UVC radiation *in vitro* brings about subtle alterations in their immunologic capabilities. Lindahl-Kiessling and Safwenberg⁷³ reported that short-term exposure to UV radiation made cells unable to respond to stimulation with mitogens. Moreover, when UV-irradiated human lymphocytes were employed as stimulators in a mixed lymphocyte reaction, stimulation of allogeneic lymphocytes failed to occur as well. Following on this work, Lafferty, Misko, and Cooley⁷⁴ demonstrated that UVC irradiation of BALB/c spleen cells of BALB/c mice *in vitro* would prevent allogeneic stimulation of responder lymph

node cells of C57BL/6J mice as assayed by cytotoxicity. Despite this inhibition of function, the UVC radiation did not affect the expression of alloantigen on the surface of irradiated cells as determined by antibody absorption. Furthermore, the capacity to generate cytotoxic lymphocytes directed against the UVC-irradiated cells from BALB/c mice could be reconstituted by providing a simultaneous allogeneic stimulation by unrelated cells (spleen cells from CBA/H mice). These observations suggested that UVC-irradiated lymphoid cells lack an accessory cell population that is essential for the induction of this immune reaction *in vitro*.

In subsequent studies, UVC radiation has been employed to inactivate the allogeneic cells responsible for the *in vitro* stimulation of cytotoxic lymphocytes, while simultaneously preserving the antigens that serve as targets for such lymphocytes.⁷⁵⁻⁷⁷ More recently, Slater et al.⁷⁸ irradiated lymphocyte populations with a UVC germicidal lamp, employing a dose of 1.2 to 1.3 mJ/cm². Although this dose failed to alter the ability of antibodies to detect HLA-A,B, or DR antigens, it inhibited the capacity of the irradiated lymphocytes to respond to mitogens and stimulate allogeneic lymphocytes. Since no suppressor activity was detected in mixing studies, the investigators concluded that antigen processing or presentation was being interrupted directly by UVC irradiation of the cells. These observations, taken as a whole, suggest strongly that UVC irradiation produces selective alterations in the cell surface determinants that are important for immunologic recognition. What is not clear in all cases is whether the target of the radiation is the lymphocytes or the antigen-presenting accessory cells contained within the population, or both.

With respect to *in vivo* irradiation, accumulating evidence now indicates that relatively high doses of UVB radiation will interrupt antigen presentation by cells obtained from organs other than skin. Greene and his associates⁷⁹ worked with the system of TNP-derivatized adherent spleen cells that will induce delayed-type hypersensitivity when injected subcutaneously into genetically identical recipients. They observed that adherent spleen cells taken from UV-irradiated mice and then haptenated were not able to sensitize UV-irradiated recipients efficiently for delayed-type hypersensitivity, as measured by injection of TNP-conjugated spleen cells into the footpad. In contrast, when the adherent spleen cells were taken from normal mice, derivatized, and injected into UV-irradiated mice, a normal delayed-type hypersensitivity response was induced. This finding suggests that the exposure to UV radiation did not affect the T cells that are responsible for the reaction but rather inhibited only the capacity of the adherent spleen cells to present the antigen. The greatest effect of UV radiation was observed when both the donors of the splenic macrophages and the recipient mice were irradiated. Injection of derivatized adherent cells from UV-irradiated donors into untreated recipients induced an intermediate level of sensitivity, whereas derivatized adherent spleen cells from unirradiated donors induced a

high level of sensitivity. Consequently, the complete inhibition of sensitization requires irradiation of both the antigen-presenting cell donor and the recipient. A significant finding from this study was that this inhibition of sensitization was associated with the appearance of antigen-specific suppressor T lymphocytes in the spleens of the recipient mice. T lymphocytes taken from mice receiving both UV radiation and haptenated cells from UV-irradiated donors could adoptively transfer that unresponsiveness to untreated animals. Studies by Letvin et al.⁸⁰ also demonstrated that adherent spleen cells taken from UV-irradiated mice also were unable to present antigen for antibody formation *in vitro*.

In further studies with this system, Fox et al.⁸¹ tested the effect of exposing antigen-presenting cells directly to UV radiation. They found that splenic adherent cells irradiated *in vitro* with 1500 J/m² of UV radiation from FS40 sunlamps and then derivatized with TNP were unable to immunize UV-irradiated mice for delayed-type hypersensitivity. Again, this failure of sensitization was associated with the induction of antigen-specific suppressor T lymphocytes in the spleen. Inherent in this model, however, is the same problem in interpretation as is encountered in the studies of Langerhans cells. That is, it is not clear whether the alteration in the immune response results from an active alteration in the antigen-presenting cells produced by direct exposure to UV radiation, or whether it results from bypassing this mode of sensitization because of inactivation of the cells by UV radiation. It is also not clear whether the alteration in antigen presentation induced by direct irradiation of the adherent cells is related to the alteration detected using cells obtained from UV-irradiated mice. Although it has been suggested that the latter alteration could occur by means of direct irradiation of circulating blood monocytes within superficial capillaries,⁸² there is no evidence for this hypothesis and other explanations are equally plausible. Also pertinent to this issue is the finding of Letvin et al.⁸³ that the adherent spleen cell populations isolated from UV-irradiated mice have fewer Ia-bearing cells than those obtained from an unirradiated animals. Furthermore, this decrease in Ia-positive cells was associated with decreased functional capabilities of these antigen-presenting cells.

Whether or not these alterations in antigen presentation are related to the systemic suppression of CHS by high doses of UVB radiation is not entirely clear. Recent work by Noonan et al.⁵⁹ provides circumstantial evidence for such an association, by demonstrating similarities in the time course and photobiologic characteristics of these two phenomena. In addition, both phenomena are associated with the induction of antigen-specific suppressor T lymphocytes. Whether or not they are related, the extraordinary aspect of this process is that UV radiation delivered to the skin will perturb in an important way the immunologic function of cells in a remote organ. It is also intriguing and probably significant that both the local and the systemic effects of UV radiation on CHS may be mediated by alterations in antigen presentation, although different antigen-presenting cell populations seem to be affected in the two cases.

9.4. Effects of UV Radiation on Other Immune Responses

Studies of the systemic effects of UV radiation on the immune responses of mice were a logical extension of the finding that UV-irradiated mice were unable to reject UV-radiation-induced skin tumors.⁸⁴ In determining the reasons why these highly antigenic tumors escaped immunologic rejection, it was important to establish the specificity of the immunosuppression in UV-irradiated mice. Clues to the mechanism of this effect of UV irradiation could be obtained by knowing whether the rejection of UV-induced tumors was the only immune response affected, whether all tissue rejection reactions were impaired, whether both T- and B-cell-mediated responses were affected, etc. In each case, a different cell or step in an immunologic pathway would be implicated as the target of the effect of UV radiation. To this end, surveys were performed of the immunologic capabilities of UV-irradiated mice during the latent period before primary skin cancers appeared.

9.4.1. Allogeneic Reactions

At present, there is no evidence of abnormality in the immune response to allogeneic tissues of mice exposed to high doses of UV radiation. This has been measured *in vivo* by the rejection of skin grafts across both strong⁵⁸ and weak⁸⁵ histocompatibility barriers, and the rejection of tumor allografts⁸⁴ by mice irradiated with UV radiation from FS40 sunlamps or an intermediate pressure mercury lamp. Although lymphocytes from UV-irradiated donors cannot mediate the rejection of UV-radiation-induced syngeneic tumors *in vivo*, either when injected systemically⁸⁶⁻⁸⁸ or when admixed with tumor cells and injected subcutaneously⁸⁹ in immunodeficient recipients, these cells can induce a local graft-versus-host reaction in F₁ hybrid recipients⁵⁸ and can mediate the rejection of allogeneic tumors in an immunodeficient host.^{86,88}

In vitro assays of allogeneic reactivity have produced similar results. The cytotoxic activity of mixed lymphocytes from UV-irradiated mice against allogeneic cells is indistinguishable from the reaction observed with lymphocytes obtained from unirradiated animals,⁹⁰ in contrast to the results when the lymphoid cells are exposed directly to UV radiation *in vitro*. The cytotoxic activity of lymphocytes against allogeneic cells *in vitro* also appears to be unaffected by prior chronic exposure of the lymphocyte donor to UV radiation *in vivo*. When UV-irradiated mice are immunized *in vivo* and their lymphocytes are restimulated and assayed *in vitro* for cytolytic activity, the response to syngeneic UV-induced tumors is impaired,^{91,92} but the response to allogeneic cells is of normal magnitude.⁹² The generation of primary cytotoxic T lymphocytes *in vitro* against alloantigens also is unaffected by prior UV irradiation of the lymphocyte donor.^{90,92}

Allogeneic responses of mice after PUVA treatment have not been examined. However, one study using PUVA in rabbits demonstrated that exposure of a skin graft site both before and after grafting prolonged the survival of allografts. Comparable treatment with UVB radiation did not affect graft survival.⁹³

9.4.2. Other Antigens

One of the more interesting tests of immunologic function in UV-irradiated mice is the ability to respond against syngeneic tumors that are induced by agents other than UV radiation. This ability is an indication of the degree of specificity of the UV-radiation-induced suppressor cells in UV-irradiated mice. In vivo tests have been carried out with three spontaneous fibrosarcomas, one spontaneous plasmacytoma, four methylcholanthrene-induced fibrosarcomas, two mammary adenocarcinomas of viral origin, and five skin tumors induced by PUVA.^{85,94} The growth of these tumors in UV-irradiated mice is indistinguishable from their growth in unirradiated animals. This strongly suggests that the suppressor cells present in UV-irradiated mice are highly specific for the antigens that occur on UV-radiation-induced tumors.

In vitro cytotoxicity tests comparing the reactivity of lymphocytes from UV-treated mice against syngeneic UV-radiation-induced and non-UV-radiation-induced tumors have not been carried out. However, some studies using TNP-conjugated syngeneic spleen cells as the immunogen have been performed. The induction of a primary in vitro cytotoxic response to TNP-syngeneic cells occurs similarly with lymphocytes from UV-irradiated and normal mice,^{85,90} as was the case with the response to allogeneic cells and to UV-radiation-induced syngeneic tumors. Psoralen plus UVA radiation treatment of mice was reported to inhibit the rejection of UVB-radiation-induced syngeneic tumors,⁹⁵ but this finding could not be confirmed in studies using a UVA source that was filtered to remove contaminating UVB radiation.⁹⁶ Whether PUVA treatment alters the immune response to PUVA-induced tumors is not known.

A few other immune reactions have been compared in UV-irradiated and normal mice and found to be normal. These include the responses of T and B lymphocytes to mitogenic stimuli in vitro, the antibody responses to sheep erythrocytes and polyvinylpyrrolidone, the inflammatory response to irritants, and the ability of activated macrophages to kill tumor cells in vitro.⁹⁷ However, one reaction that is suppressed in UV-irradiated mice is their ability to serve as recipients in a local graft-versus-host assay.⁵⁸ Injection of parental lymphocytes into the footpads of UV-irradiated F₁ hybrid mice produces less enlargement of the popliteal lymph node than occurs in unirradiated recipients. This suppression is transient, and its time course parallels that of the systemic suppression of CHS in UV-irradiated mice.

Whether delayed-type hypersensitivity to antigens other than those used in CHS is suppressed in UV-irradiated mice is not known. However, both PUVA and UVB treatment of guinea pigs decreased the delayed-type hypersensitivity reactions to dinitrophenol-conjugated bovine gamma globulin. Suppression of the reactions elicited both in irradiated and in untreated skin was observed, indicating that both local and systemic effects of the treatments had occurred.⁹⁸

9.5. Significance of the Experimental Studies

It is apparent from the studies carried out so far that exposure of laboratory animals to UV radiation or to PUVA can affect profoundly certain types of immunologic reactions. In addition to its effects on tumor rejection in mice, treatment with UV radiation appears to interfere with both the induction and the expression of CHS reactions. Not all cell-mediated immune reactions are affected by UV radiation, however. The development of cytolytic T lymphocytes and the rejection of allogeneic tissues appear to be unaltered by exposure of animals to UV radiation, although this may not be the case with PUVA treatment. We do not know to what extent immunologic reactions are impaired in the UV-irradiated host, and additional studies of other types of immune reactions, such as delayed-typed hypersensitivity to protein antigens, and susceptibility to intracellular microorganisms are needed.

What is clear, however, is that certain, specific immunologic pathways are selectively impaired by UV irradiation of animals. Even though there are still many uncertainties in our understanding of how UV radiation causes these alterations *in vivo*, the realization that such effects can occur has stimulated considerable interest in the implications of these findings for humans. In addition to their potential practical applications, these findings are important for increasing our understanding of the mechanisms that govern immunologic responses. Much of the evidence concerning the ways by which UV radiation alters immune function points to a prominent role of the antigen-presenting cells. Since there is little information available on how and where antigen presentation occurs *in vivo*, the suggestions that UV radiation can alter selectively the presentation of certain antigens provide a new approach for investigating this important problem in immunobiology. In addition, the evidence that suppressor mechanisms may be activated as a consequence of these UV-radiation-induced alterations in antigen presentation is potentially of considerable significance in advancing our understanding of the immunologic regulatory system.

9.6. References

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Chapter 10

Effect of UV Radiation on Immune Reactions in Humans

Warwick L. Morison

10.1. Introduction

The possibility that exposure to nonionizing radiation might influence immune function in man was first considered in the early part of this century. However, although there has been interest in this area of research for a long time, progress has been very slow. The likelihood is that exposure to UV radiation can alter cells of the immune system and inhibit certain immune responses, but thus far, most reports on this subject have been descriptive, limited in scope, and have not addressed the mechanisms or the significance of UV-radiation-induced changes. Historically, there has been interest in the possibility that exposure to sunlight might be of benefit in controlling infectious diseases. Exposure to sunlight and artificial sources of radiation was reported originally to aid in the treatment of tuberculosis and pertussis. Weekly exposures to a mildly erythemagenic dose of UV radiation decreased the incidence of respiratory tract infections during the winter months in one group of adults compared with a control group.¹ Children given a much larger dose of radiation, however, had an incidence of such infections similar to that in a control population.² Except in the U.S.S.R., studies of this type have not been repeated or extended in recent years, but in that country there is considerable interest in the beneficial effects of UV radiation in both health and disease.³ Regular exposure of several thousand children to UV radiation during the winter months was claimed to have a beneficial effect mediated by the immune system. However, the nature of the beneficial effect and the evidence for involvement of the immune system are unclear.

Interest in human photoimmunology has been reawakened in recent years due to two findings. (1) The demonstration that UV radiation can influence immune reactions in animals has, of course, aroused interest in whether humans

might show similar changes; and (2) the widespread use of UV radiation in the treatment of disease, including some diseases thought to have an immune pathogenesis, has raised the question of whether some of the beneficial or adverse effects of these treatments might be mediated by an alteration in immune function. Most interest has focused on the effects of psoralen plus UVA radiation (PUVA) therapy, probably because UVA radiation is more penetrating than the shorter wavelengths of the UV spectrum. The effects of therapeutic regimens of PUVA and UVB radiation on immunity in patients with various diseases are discussed elsewhere in this book. For this reason, the studies reviewed here are restricted mainly to those carried out with normal subjects.

10.2. In Vivo Immune Responses

There are only a few reports on the effects of UV radiation on delayed-type hypersensitivity reactions. In one, a brief exposure to UVC radiation at the site of intradermal injection of streptokinase-streptodornase diminished the response to this antigen in a single, previously sensitive subject.⁴ A diminished ability to elicit a contact hypersensitivity (CHS) reaction to dinitrochlorobenzene (DNCB) has been reported in sun-damaged skin, compared to adjacent undamaged areas.⁵ Recently, Halprin et al.⁶ attempted to use UV radiation to prevent the induction of CHS to nitrogen mustard. This chemotherapeutic agent is effective in controlling cutaneous lymphoma and psoriasis in humans, but most persons eventually develop CHS to the compound. In this preliminary study, both treatments with PUVA and UVB radiation were reported to retard the onset of contact allergy to nitrogen mustard.

The mechanisms by which UV radiation inhibits the development of these reactions in humans are unknown. However, as a consequence of the observations that have been made in animals, there has been renewed interest in the effects of UV radiation on Langerhans cells in human skin, because of the possible relevance of such effects for CHS. Some years ago, the observation was made that daily exposure to sunlight for a period of 8 wk reduced the number of Langerhans cells in human skin.⁷ Furthermore, daily exposure of human skin to 6 minimal erythema doses for 2 wk resulted in elimination of all recognizable Langerhans cells from the skin, as evaluated by electron microscopy. More recently,⁸ exposure of human skin to moderate doses of either UVA or UVB radiation was found to result in a marked alteration of the surface membrane properties of these cells; both ATPase activity and Ia antigens were found to be markedly reduced. Electron microscopy revealed that many of the Langerhans cells were still present, but they showed evidence of damage at doses that resulted in virtually complete loss of the membrane markers. In certain dose ranges, Langerhans cells were the only epidermal cells to display morphologic damage at the ultrastructural level, which suggests that these cells are especially sensitive to

UV radiation. In these studies, there have been no attempts to correlate these alterations in Langerhans cells with the capacity of the skin to support induction or elicitation of CHS. However, in one preliminary report,⁹ the investigators reported that skin with chronic actinic damage had a decreased number of Langerhans cells, as identified by ATPase staining, compared with unexposed skin from the same individual. This finding may be related to the observation of O'Dell et al.⁵ that the elicitation of CHS to DNCB is impaired in sun-damaged skin compared to adjacent unexposed areas.

At present, these limited observations only suggest that UV radiation may inhibit the development and/or the expression of CHS in human skin. Furthermore, although UV-radiation-induced alterations in Langerhans cells have been documented, there is as yet no firm evidence that these alterations are causally related to functional alterations in immune responses. Additional studies are required to define the effects of UV radiation on immunity in humans, to determine whether such effects are exerted locally at the site of exposure and/or systemically, and to delineate the mechanisms involved.

10.3. Effects of UV Radiation on Lymphocytes

One of the first topics to attract interest in the area of human photoimmunology was the effect of UV radiation on circulating blood leukocytes. By means of cell counts and examination of blood smears, Laurens¹⁰ concluded that acute or chronic exposure to radiation increased the number of lymphocytes in the peripheral blood. These studies are somewhat difficult to interpret because various radiation sources were used, and both healthy subjects and patients with disease were examined. This issue has been reexamined more recently by Morison et al.¹¹ Normal subjects were given a single, whole-body exposure to UVB radiation which produced a mild or a marked erythema. The number and *in vitro* activation of lymphocytes from these subjects was monitored over a subsequent 72-hr period. Shortly after exposure to UV radiation, the subjects who developed marked erythema had a decreased proportion of circulating cells capable of forming E rosettes, a marker for T lymphocytes. This decrease was most pronounced at 8 to 12 hr after exposure, and the proportion of T lymphocytes returned to normal levels by 72 hr. Accompanying this decrease was a corresponding increase in the proportion of lymphocytes without T- or B-cell markers (null cells), but there was no significant change in the proportion of B lymphocytes. The decrease in T lymphocytes was paralleled by a decrease in the response of the circulating cells to phytohemagglutinin (PHA), which is a T-cell mitogen. Subjects who developed only a mild erythema did not show this decrease in reactivity to PHA, suggesting that this effect might be related to the dose of UV radiation. However, both groups showed a marked increase in the number of polymorphonuclear leukocytes.

The effect of PUVA treatment on lymphocytes in humans has attracted some interest recently. The proportion of circulating lymphocytes forming E rosettes decreased after 4 PUVA treatments, but returned to normal levels after 8 treatments in one study.¹² In contrast, no such alteration was observed in another study.¹³ In a third study, a single PUVA treatment in which the whole body was irradiated was given to a group of normal subjects and serial examinations of peripheral blood lymphocytes were performed.¹⁴ The doses of radiation were selected so that marked erythema was produced in some subjects and minimal erythema was produced in others. In subjects exhibiting marked erythema after PUVA treatment, a decrease in the proportions of circulating T and B lymphocytes was observed, along with a corresponding increase in the proportion of null cells. There was no alteration in the absolute number of circulating lymphocytes. Doses of PUVA that resulted in minimal or no erythema had similar but less marked effects on lymphocytes. These changes were detectable 30 min after exposure, were maximal 12 to 16 hr later, and returned to pretreatment levels by 72 hr after irradiation. The response of the lymphocytes to stimulation with PHA was unaffected, as was the absolute number of circulating polymorphonuclear leukocytes. These last two findings contrast with those observed after exposure to UVB radiation in a parallel study.¹¹

The mechanism of these changes in peripheral blood leukocytes following exposure to UV radiation is unknown. Alterations in the cell membrane may explain the inability of lymphocytes to be identified by surface markers and also may explain the decreased response of the cells to mitogen stimulation. Alternatively, sequestration of damaged cells in internal organs and an influx of immature (null) cells could explain these phenomena.

10.4. In Vitro Exposure of Lymphocytes to UV Radiation

In contrast to the paucity of literature on the effects of in vivo exposure to UV radiation on lymphocytes, there are many studies that have addressed the effects of in vitro exposure on the activity of human lymphocytes. UVC radiation has received the most attention, but more recently, PUVA treatment has been examined in some detail also. The source of lymphocytes used in these studies has varied, but human peripheral blood mononuclear (PBM) cells have been employed most frequently. In vitro irradiation of these cells causes a dose-dependent loss of viability, as measured by trypan blue dye exclusion.¹⁵ UVC radiation kills more cells per unit energy than UVB radiation, which in turn kills more cells per unit energy than UVA radiation. The lethal dose of the three wavebands is in an approximate proportion of UVC:UVB:UVA = 10:10:10⁵.

Human T lymphocytes were reported to be more sensitive to UVC^{16,17} and UVB radiation¹⁶ than were B lymphocytes. However, a recent study found that

T and B lymphocytes exhibited a similar degree of sensitivity to UVC radiation, both in terms of the number of cells killed and the rate of killing.¹⁸ The sources of human lymphocytes and the time of testing after exposure to radiation varied in these studies, which may explain the differing results. The addition of psoralens greatly increased the toxicity of UVA radiation for both human PBM cells¹⁹ and a lymphoblastoid cell line.²⁰ Pyknotic changes in the nuclei and possible holes in the cell membrane were observed in an ultrastructural study of lymphocytes following PUVA treatment *in vitro*.²¹

Human PBM cells treated with UVC radiation *in vitro* lose their ability to stimulate allogeneic lymphocytes in the mixed lymphocyte culture reaction.²² The ability of the irradiated cells to respond to PHA was inhibited also, although viability, as determined by trypan blue dye exclusion, was not affected in the dose range required to produce these alterations. These results are similar to those described for murine lymphocytes, although the analyses with human cells are somewhat less detailed. Psoralen plus UVA radiation treatment of either stimulator or responder cells in the mixed-lymphocyte-culture reaction also can lead to an inhibition of the proliferative response.^{23,24}

The ability of human PBM cells to incorporate tritiated thymidine into DNA after stimulation with PHA is reduced after exposure of the cells to UV radiation *in vitro*.²⁵ This reduction is dose and wavelength dependent: UVC radiation is more effective than UVB radiation, which, in turn, is more effective than UVA radiation. The relative sensitivity of the cells to the three wavebands is similar in proportion to that observed when viability was measured. However, it is interesting that viability, as determined by trypan blue dye exclusion, seemed to be affected by lower doses of UV radiation than those required to suppress mitogen responsiveness. This finding suggests that trypan blue dye exclusion may not be an accurate indicator of cell survival after exposure to UV radiation. Psoralen plus UVA radiation treatment also decreases the response of PBM cells to PHA^{26,27} in a dose-dependent manner. The response to two other mitogens, concanavalin A, which stimulates T lymphocytes, and pokeweed mitogen, which stimulates B lymphocytes, were suppressed by doses of PUVA that were very similar to those found to affect the PHA response.¹⁹ Furthermore, in contrast to the findings with UV radiation alone, viability was altered by higher doses than those required to suppress mitogen responsiveness.

Recently, Levis, Lincoln, and Dattner²⁸ have studied the antigen-presenting activity of haptenated human PBM cells in an *in vitro* proliferation system. Lymphocytes obtained from hapten-sensitive subjects were stimulated with haptenated PBM cells. Exposure of the haptenated cells to UVC radiation *in vitro* decreased their ability to stimulate proliferation of the lymphocytes. However, the mechanism of this effect has not been determined.

In vitro exposure of lymphocytes to UV radiation also can affect chromosome structure and can induce DNA repair. Unscheduled DNA synthesis, thought to represent the repair of damaged DNA, occurs in PBM cells after exposure to

UVC radiation; this repair synthesis is essentially complete after 6 hr.²⁹ Autoradiography shows that the sites of repair synthesis are not distributed randomly within the nucleus, but are confined largely to the area adjacent to the nuclear membrane.³⁰ Furthermore, the distribution of the sites at which repair occurs is not related to the distribution of euchromatin or heterochromatin within the nucleus. Differences in the ability of human lymphocyte subpopulations to repair DNA have been reported. B lymphocytes seem to be much more efficient in repairing DNA damaged by UVC radiation than T lymphocytes.^{17,18} However, this apparent difference may be attributable to differences in the size of the unlabeled intercellular nucleotide pool, because the repair capacity of isolated DNA from the two types of cells appeared to be similar.¹⁷

The effect of UV radiation on chromosomal structure of lymphocytes does not appear to have been studied. However, PUVA treatment of lymphocytes in vitro has been found to increase chromosomal aberrations³¹ and the frequency of sister-chromatid exchanges³² in a dose-dependent manner.

10.5. Significance

Most of the studies in normal human subjects of the effects of UV radiation and PUVA on immunity have been necessarily short-term and descriptive. Even so, however, such treatments clearly can affect certain elements of the immune system, and there is at least a suggestion of perturbations in immunologic function as well. The alterations in Langerhans cells and peripheral blood leukocytes that have been observed after in vivo treatment with UV radiation provide concrete evidence that nonionizing radiation interacts, either directly or indirectly, with cells of the immune system. However, the significance of these changes for immunologic functions is still ill-defined. Another issue that cannot be addressed easily using normal human subjects is the effect on the immune system of repeated UV radiation treatments, as opposed to single or few exposures. Most likely, information on these topics will come from studies in experimental animals in which it is possible to correlate changes in immunologic function in vivo with these cellular alterations, and from studies on patients treated chronically with UV radiation for therapeutic purposes.

The amount of information available on the effects of UV radiation on immune function in humans is very limited at present, and the studies reported to date cannot be considered definitive by any means. However, these studies are interesting in that they parallel, in some respects, the findings in laboratory rodents, raising the possibility that UV radiation may be capable of modulating certain immune responses in humans. This prospect is an exciting one because it suggests that it may be possible to control undesirable immune reactions in humans by judicious application of the new findings on UV irradiation of ex-

perimental animals. Even though there is little experimental evidence to support the hypothesis that UV radiation can affect immunologic function in humans, additional explorations in this area clearly are warranted.

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Section III

Clinical Photoimmunology

The possibility that photons may induce or alter immune responses was first suggested by observation of various diseases, all of which are characterized by photosensitivity. The term *photosensitivity* is used here in a clinical sense and may be defined as an altered reaction of the skin or other organs following exposure to nonionizing radiation. The altered reaction may be in the form of an accentuation of the normal responses or development of an abnormal response. Thus, photosensitivity may be expressed as a decreased threshold for the development of UV-radiation-induced erythema, an abnormal time course for such erythema, the development of a rash or other abnormal reaction in the skin, or the malfunction of systemic organs.

Photosensitivity is a clinical feature of a variety of cutaneous and systemic diseases. Involvement of photons in the pathogenesis of photosensitivity disorders is usually obvious from the clinical features of the patients and can be confirmed by provocative testing with sunlight or artificial sources of radiation. Immunologic mechanisms have been postulated to explain the etiology and/or pathogenesis of a number of these disorders, but have been demonstrated in only a few. Much more investigation is required to confirm that alterations of immune function are involved in these diseases and to determine how radiation influences immune function at a cellular and molecular level. The selection of diseases for inclusion in this section has been restricted to conditions in which there is reasonable evidence that a specific interaction between photons and immune responses is involved in their pathogenesis or, alternatively, in which much investigation has been conducted with that possibility in mind.

Solar urticaria, which is simply the development of hives following exposure to sunlight, was the first disorder of photosensitivity to be categorized as immunologic or “allergic” in nature. The suggestion was based on the belief that all urticaria had an immune pathogenesis which is a concept that is no longer accepted. However, there is reasonable evidence that some cases of solar urticaria do represent an immune response triggered by exposure to nonionizing radiation. In other cases, there is no evidence for an immune response and a search for some other mechanism may be a fruitful area of research.

In the late 1930s, the term *photoallergy* was first introduced to describe some cases of photosensitivity following exposure to exogenous chemicals. It was suggested that photoallergy represented a state of specific altered reactivity and that it had an immune pathogenesis. In this condition, eczema develops in the skin at the site of interaction between a chemical and nonionizing radiation, and most evidence points toward this being mediated by a cellular immune response. However, there are many instances in which a chemical has been labeled as a photoallergen without any convincing evidence that the reaction it produced in combination with radiation had an immunologic basis. In addition, there are other diseases such as photosensitive eczema and actinic reticuloid that may represent a photoallergic response, but at present, clinical suspicion is the main evidence for this possibility. In these diseases, the clinical features suggest an association with photoallergy but a photoallergen has not been identified.

The autoimmune diseases that are associated with photosensitivity differ from other disorders thought to have a photoimmunologic pathogenesis in that more is known of the immunologic aspects than the photobiologic aspects of these diseases. In lupus erythematosus, pemphigus, and pemphigoid, a reasonably well-defined immune pathogenesis has been established. At times, these diseases can be induced or exacerbated by exposure to nonionizing radiation; however, it is not at all clear how photons interact with the immune responses that mediate these disorders.

In addition to a role for photon-induced alterations of immune responses in the pathogenesis of disease, it has recently been suggested that such alterations may be responsible for the beneficial effect of UV radiation in the treatment of various skin diseases. Furthermore, it is also possible that some of the adverse effects observed following such treatment may result from an interaction of radiation with the immune system. Both these possibilities are still at a hypothetical stage of development, but they may represent the most interesting aspect of the future of clinical photoimmunology.

W.L.M.

Chapter 11

Solar Urticaria

Warwick L. Morison

11.1. Introduction

Solar urticaria is a disease in which a person develops hives during or immediately after exposure to nonionizing electromagnetic radiation contained in the solar spectrum. The urticaria is restricted to the site of exposure, but systemic symptoms such as headache and malaise can accompany it. Solar urticaria was first described as a distinct clinical entity in 1905 by Ward,¹ who reported the case of a woman who developed an urticarial reaction and associated systemic symptoms following exposure to sunlight. By conducting some simple tests, he found that the reaction in this patient was triggered by exposure to UVA radiation and that other physical stimuli such as cold, heat, and X rays did not reproduce the reaction. The name solar urticaria was coined by Duke² in 1923, when he described another case and compared solar urticaria to urticarial reactions precipitated by other physical stimuli. Since those early reports, many cases of solar urticaria have been described and a large body of literature on its photobiologic aspects has been published. A few studies have been made of the possible immunologic features of the disease and the results suggest that, in some cases, the pathogenesis may involve an interaction between nonionizing radiation and the immune system.

11.2. Etiology

Solar urticaria is usually idiopathic. Only in rare cases has there been a suggestion of some underlying cause, but the possibility that an exogenous or endogenous photosensitizer might trigger the disease has attracted much interest and speculation. Several therapeutic agents have been associated with an urticarial reaction following exposure to solar radiation. However, unlike idiopathic solar

urticaria, these reactions have included other features apart from hives and pruritus, and there is little difficulty in distinguishing them as separate entities. Reports of factory workers exposed to pitch have suggested that this agent can cause solar urticaria.³ When subsequently exposed to radiation (340 to 440 nm), they developed an initial “smarting” sensation in the skin, immediate erythema and edema, and delayed erythema and pigmentation. Again, this syndrome, presumably caused by a phototoxic reaction to pitch, is not easily confused with idiopathic solar urticaria because the latter does not include a delayed phase of erythema and pigmentation. The possibility that a disturbance of porphyrin metabolism may present as solar urticaria has been considered in many case reports. The first description of erythropoietic protoporphyrina⁴ was of a patient who presented with solar urticaria, but this association appears rare because no further reports have appeared. Therefore, if an exogenous or endogenous photosensitizer plays a role in triggering typical cases of solar urticaria, it has yet to be identified.

Certain diseases are associated with solar urticaria. The combination of solar urticaria and another idiopathic photodermatosis, polymorphic light eruption (PMLE), is not uncommon. However, since even less is known of the pathogenesis of PMLE than of solar urticaria, this observation does not advance our understanding of the etiology of solar urticaria to any great extent. Very rarely have other diseases been reported in association with solar urticaria. One patient presented with solar urticaria that had developed during a long history of a multi-system disorder, which was probably a benign form of systemic lupus erythematosus (SLE).⁵ Photosensitivity, urticaria, and SLE was the subject of another report,⁶ but the urticaria was apparently not triggered by sun exposure. Thus, solar urticaria and SLE do not appear to be associated except as could be explained by chance. Solar urticaria has been reported in several patients with cutaneous lymphocytoma,⁷ a quite rare condition; this seems to be a real association, although its significance is not immediately obvious. It is interesting that patients with this condition may also develop PMLE, which may be further evidence for an association between these two photodermatoses.

11.3. Clinical Features

Solar urticaria usually first appears in young adult life, but it can commence at any age. It is more common in females, with a female/male ratio of about 3:1, and it has been reported worldwide. No geographic or racial predominance or sparing has been noted.

The clinical picture is remarkably uniform among cases. Symptoms and signs appear within minutes of exposure to sunlight or the eliciting wavelengths and a latency period of more than 15 min is unusual. The patient first complains

of tingling or pruritus of the skin in the exposed area. This is accompanied or soon followed by erythema. A wheal, superimposed on the erythema, appears a few minutes later. The reaction gradually subsides with disappearance of the wheal and later the erythema, but the total duration of the eruption seldom exceeds a few hours. The urticarial reaction is largely confined to the site of exposure and lesions do not occur at distant, nonexposed sites. However, when a clearly defined area is exposed, a flare reaction (up to 2 cm beyond the field of exposure) accompanies appearance of the wheal. Individual lesions may be small (~ 1 cm in diameter), or they may form as large sheets occupying the entire exposed area. Marked systemic symptoms are uncommon, although mild headache, dizziness, and malaise often accompany an extensive reaction. Syncope can occur as a result of hypovolemia, and this can be potentially lethal when sun exposure is combined with swimming.

11.4. Diagnosis

The diagnosis is usually made readily on the basis of the history, provided the patient accurately reports the immediate onset and short duration of the reaction and is able to observe the appearance of hives. Of course, it is essential to actually see the eruption because the symptoms can be confused with eruptions caused by heat and with immediate erythema responses. When a patient has both solar urticaria and PMLE, a historical diagnosis is difficult because one reaction tends to lead into the next and the patient is likely to report the total duration of symptoms. In such a patient, a diagnosis of solar urticaria is readily confirmed by reproducing the eruption: Sunlight is the most convenient source of radiation in most situations, and a short exposure of a limited area of the body is all that is required. The rare case of erythropoietic protoporphyrria that presents as urticaria can be diagnosed by determining the amount of protoporphyrin in red blood cells. It is necessary to determine the wavelengths responsible for urticaria and the minimal urtication dose of radiation and to perform an antinuclear antibody test if phototherapy or photochemotherapy is being considered as treatment.

11.5. Prognosis

The prognosis in solar urticaria is variable. The disease persists for many years in most cases and possibly for life, but there have been no prospective studies to evaluate this aspect of the condition. Patients often report considerable variation in sensitivity over time and some undergo a progressive loss of sensitivity during the course of a summer. However, this "hardening" phenomenon is confined to patients that can tolerate at least brief exposures to sunlight, whereas

the most severely affected patients live a hermit-like existence of almost totally avoiding exposure to the sun.

11.6. Treatment

The treatment of solar urticaria is very difficult, as evidenced by the variety of therapies that have been evaluated. Patients sensitive to only UVB radiation and afflicted by mild disease can control reactions by using sunscreens and avoiding excessive exposure to the sun. However, sunscreens are seldom effective in patients with severe disease, and total avoidance of sunlight has been suggested as the most effective alternative because even patients sensitive to the whole range of visible radiation seldom develop urticaria under room lighting.

Considerable progress has been made in the use of therapeutic approaches that are somewhat less socially disturbing. Duke,² in 1923, noted that in his patient, repeated exposure to radiation, including the action spectrum for urticaria, resulted in a progressive loss of sensitivity. This phenomenon has been observed many times since then, and occasionally it has been used to therapeutic advantage. Rubin, Beal, and Rothman⁸ in 1947, treated a patient with an antihistamine (which produced some increase in sunlight tolerance) plus repeated exposures of half of the body to the eliciting waveband. The irradiated areas of the body showed a two hundredfold greater increase in tolerance to radiation as compared to nonirradiated areas.

Ramsay used a variation of this therapeutic approach, which he called *induction of tolerance* so as not to presuppose the mechanism.⁹ Eight of nine patients successfully responded to his treatment. Small areas of the body were repeatedly exposed to radiation that included at least part of the spectrum responsible for urticaria, and when tolerance had been established, the frequency of exposure was progressively reduced. Various regions of the body were treated until tolerance was induced over the whole body. However, reactivity returned after 48 to 72 hr, necessitating whole-body exposures to radiation every 24 hr. Not surprisingly, some patients found the treatment more troublesome than the disease, and long-term maintenance therapy was not feasible in most cases. The mechanism of this therapy is presumed to be depletion of a mediator or some other reactant involved in the urticarial response. A possible site for this effect is the mast cell. Degranulation of mast cells has been observed in patients with solar urticaria after exposure to radiation, and histamine and other mediators present in mast cells may produce the urticarial response.¹⁰ Repeated exposure to radiation may keep the mast cells constantly depleted of mediators.

Another variation on this therapeutic approach is psoralen plus UVA radiation (PUVA) therapy. Encouraging results were obtained in two patients treated with PUVA therapy^{11,12}; each developed a marked increase in tolerance

to sunlight. We recently evaluated PUVA therapy in six patients and confirmed that the treatment is very successful. After 4 to 8 wk of treatment, all patients developed a tenfold or greater increase in tolerance. Two observations suggest that the mechanism of action of PUVA therapy is quite different from that of induction of tolerance by radiation. First, three of the patients in our study were not sensitive to UVA radiation and yet they responded to PUVA therapy as well as did the patients who were sensitive to this waveband. Second, sensitivity in our patients did not return quickly after cessation of treatment, and a single weekly exposure to either sun or PUVA treatment was usually adequate for the maintenance of protection. Psoralen plus UVA radiation therapy may act by increasing the capacity of melanin and the stratum corneum to absorb solar radiation, or, alternatively, it may alter the reactivity of some cell or cellular component involved in the production of urticaria. The latter possibility is supported by a recent report that mast cells were not degranulated after tolerance had been induced by a course of PUVA therapy in several patients with solar urticaria.¹³ The level of protection in those patients exceeded the anticipated sunscreening effect of changes in the melanin-stratum corneum protective barrier. These observations suggest that PUVA may produce an alteration of mast cell function, so that the threshold for degranulation and release of mediators is greatly increased. Alternatively, PUVA may deplete mast cell mediators without causing degranulation, or it may alter the function of some other type of cell involved in the reaction.

Other treatment modalities have been tried in solar urticaria. Chloroquine has been suggested,¹⁴⁻¹⁶ but has not gained wide acceptance. Antihistamines have also been used, but the response to this therapy in patients has ranged from no effect¹⁷ to a marked reduction in the duration and severity of the urticarial reaction.¹⁸ In recent careful evaluation of these agents,¹⁹ investigators found that an H1 antihistamine was superior to an H2 antihistamine but that neither agent alone nor the combination of the two agents was very effective. We have also found that an H1 antihistamine can provide some improvement in patients with solar urticaria; however, side effects limit the tolerated dosage, and the amount of improvement is not clinically significant. Another mediator inhibitor, indomethacin, which inhibits prostaglandin synthetase, was without effect in four patients whom we studied. Oral β -carotene therapy was also found to be of no benefit in three patients with solar urticaria.²⁰

11.7. Pathogenesis

Electromagnetic radiation is clearly involved in the pathogenesis of solar urticaria as the initial step in the induction of urticarial lesions. In at least some cases, mast cell degranulation and release of mediators appears to be involved in

the final steps of the pathway. The site and nature of initial photochemical reaction and the intervening events are largely unknown.

11.7.1. Photobiologic Aspects

Most studies have reported that the wavelengths responsible for solar urticaria are in the UV-visible portion of the electromagnetic spectrum. However, the spectrum could be even broader than this. There is a report of urticaria being induced by infrared radiation,²¹ and a failure to induce urticaria with other techniques for heating the skin suggested that the reaction was akin to solar urticaria. In another patient,²² urticaria followed exposure to X rays and the reaction could be passively transferred with serum. However, the delayed onset of urticaria at 6 hr post exposure and the 72-hr duration for the reaction suggest that this syndrome is distinct from solar urticaria. Several different methods have been used to determine the wavelengths responsible for solar urticaria. Perhaps the most detailed study was that of Ive, Lloyd, and Magnus²³ who used a monochromator to determine the limits and spectral maxima for production of solar urticaria in 17 patients. On the basis of their findings, they suggested that patients could be classified into four or five groups according to the wavelengths responsible for the reaction or into three groups according to the spectral maxima. Harber et al.,²⁴ using broadband sources of radiation, studied five patients, and on the basis of their findings together with a review of previously published reports, they suggested a classification for solar urticaria based on both the wavelengths responsible for the reaction and the results of serum transfer tests. In the initial report, six groups of patients were distinguished, including patients with erythropoietic protoporphyrina. In a later report,²⁵ two more groups were added, namely patients sensitive to infrared radiation and patients with cholinergic urticaria. While it might be justified to define patients sensitive to infrared radiation as a group, despite only a single case report of such sensitivity, the inclusion of protoporphyrina and cholinergic urticaria in the classification probably only leads to confusion, since these are clearly defined entities unrelated to typical cases of solar urticaria. A recent study of the wavelengths responsible for production of solar urticaria in 12 patients confirmed previous findings that reactivity across the UV-visible spectrum varied widely.

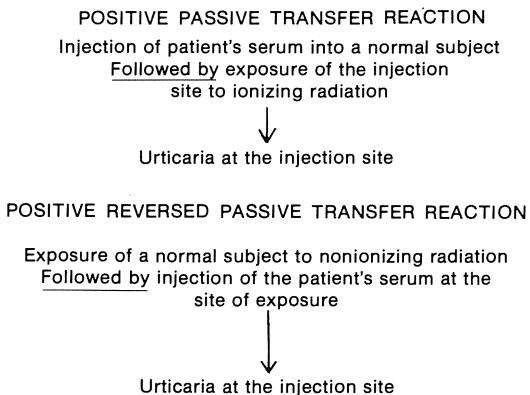
Several conclusions can be drawn from these photobiologic studies. First, the range of wavelengths of radiation responsible for production of solar urticaria is broad, covering most of the UV-visible waveband. In an individual patient, the action spectrum may be narrow or very broad. Second, spectral maxima have been found to vary considerably among patients. Third, as our group and others²³ have observed, the wavelengths responsible for the reaction in any given patient are not fixed but can vary over time. For example, a patient we have studied gave a strong urticarial response to both UVB and UVA radiation one year, but

the following year responded only to exposure to UVB radiation. The wavelength dependence of a disease such as solar urticaria is being investigated to determine the chromophore involved in a reaction. The broadness of the action spectrum for solar urticaria suggests that more than one chromophore may be involved in this disease, since most molecular species have absorption spectra that are much narrower than the width of the UV-visible spectrum.

11.7.2. Immunologic Aspects

Since the earliest descriptions of the disease, an immune pathogenesis has been suggested for solar urticaria, primarily because all types of urticaria were believed to be manifestations of an antigen-antibody reaction. Indeed, certain types of urticarias can be a manifestation of such a reaction, but this has been demonstrated mainly in urticaria caused by exogenous allergens such as food and drugs. The evidence that physical urticarias also have an immune pathogenesis is not so strong. In the case of solar urticaria, the main support for an antigen-antibody-mediated reaction is the finding that, in some cases, urticaria is transferred by serum. Presumably, the antibody responsible for the urticaria is transferred in the serum. However, there is no information on the nature of the antigen responsible for triggering production of an antibody and very little is known about the nature of the antibody, if indeed one is present in patients with this disease. Furthermore, of the types of solar urticaria described by Harber et al.^{24,25} it has been possible to demonstrate serum transfer in only two types of solar urticaria; in others, this test is reported to be negative.

Two types of serum transfer tests have been used in solar urticaria to study the nature of the reaction (Fig. 11.1). The passive serum transfer test is analogous to the Prausnitz-Kustner reaction in that the patient's serum is injected intradermally into a normal subject and the site is exposed to the eliciting wavelengths within 24 hr. A positive result is indicated by the immediate appearance of an urticarial wheal at the site of injection and exposure. Presumably, reaginic antibodies in the serum bind to mast cells, the subsequent exposure to radiation alters a normal component of the skin to form the antigen, and mast cell degranulation and release of mediators is triggered by an antigen-antibody reaction on the cell surface. A positive passive transfer reaction could also be explained by the presence of a phototoxic agent in the serum. However, this possibility can be eliminated by demonstrating a reversed passive transfer reaction (Fig. 11.1). This test consists of injecting serum intradermally into a normal subject who has been exposed to radiation. A positive reaction in this test eliminates the possibility that a phototoxic agent has been transferred in the serum. In some patients sensitive to UVB radiation, both tests are positive. However, the passive transfer test is positive in some patients who are sensitive to 400- to 500-nm radiation, although the reversed passive transfer test is negative. Recent studies^{27,28} in pa-

**Figure 11.1.** Serum transfer tests.

tients with this type of urticaria may have provided an explanation for this finding. Serum from such a patient injected into a normal subject, gave a positive passive transfer reaction but a negative reversed passive transfer reaction. When the patient's serum was exposed to radiation and then injected into the patient, an urticarial response was seen, but the same irradiated serum gave a negative result following injection into a normal subject. Irradiated serum from a normal subject gave a negative result in both the patient and the normal subject. The authors' explanation for these results was that irradiation of the patient's serum resulted in the formation of an antigen from a component that was not present in normal serum. The antigen, which was present in excess, fixed all of the antibody in the sample of the patient's serum; thus, there was no antigen-antibody reaction when the serum was injected into the normal subject. However, when the serum was injected into the patient, the remaining excess antigen reacted with the patient's own antibody. A study²⁸ of three more patients with the same type of urticaria gave essentially the same findings except that irradiated serum from a normal subject gave a positive response in one patient, indicating that the antibody in that patient could react with an antigen formed from a component of normal serum.

The nature of the antibody responsible for solar urticaria has attracted some study. Fractionation of a patient's serum with NH₄SO₄ indicated that the factor responsible for a positive passive transfer reaction was mainly in the globulin fraction.²⁴ This was confirmed in 1970 by Sams,²⁹ who used electrophoresis to examine serum from two patients. Sams' study is the most detailed examination of the nature of the antibody that has been published. By fractionation on DEAE-cellulose and Sephadex, he found that the protein was probably not IgG or IgM, but further identification of the antibody was not possible because the transfer

activity in the samples was lost. Now that IgE globulins have been characterized, that study deserves repeating because the factor responsible for the transfer is likely to be in that class of proteins. Certain features of the serum factor have been characterized: (1) It is thermolabile, and incubation at 60°C for 1 hr abolishes its activity; (2) it progressively loses activity in a passively sensitized site after exposure to radiation; and (3) it retains reactivity in the sensitized site several days after injection.^{29,30} These properties are consistent with the serum factor being an IgE reaginic antibody. However, the factor responsible for serum transfer has some properties which are not typical of an IgE antibody. Several workers have noted that the activity of serum is lost with dilution, and serum is seldom active above a dilution of 1:10. The serum factor also appears to have a short half-life; little activity could be detected in serum after storage in a refrigerator, presumably at 4°C, for 8 days³¹ or 44 days.³⁰

Polymorphic light eruption and solar urticaria commonly occur in association. Some of these patients probably have positive serum transfer tests for the urticarial reaction; however, I have found no reports of such findings and no reports of testing for passive transfer of PMLE in such patients. The wavelengths responsible for the two reactions are different in some patients.²³ This group of patients should be studied further.

It is usually presumed that the mast cell is the target for the urticarial reaction and is the source of mediators. An increased number of mast cells in the dermis of patients with solar urticaria has been reported in studies that used standard histologic techniques and staining with toluidine blue.^{18,32} This finding requires confirmation using a more sensitive technique, such as examination of 1-μm sections, which would permit more accurate quantitative analyses of the density of mast cells in the skin. In other studies, investigators observed degranulation of mast cells following exposure to radiation.^{10,13,18} Still other workers have found elevated levels of histamine in venous blood draining the site exposed to radiation^{10,13,33}; the histamine level was raised within a few minutes of exposure and returned to baseline values 20 to 30 min after exposure. A threefold to fivefold increase in eosinophil and neutrophil chemotactic factors was found to parallel the rise in histamine levels in the blood,³³ indicating that other mast cell mediators are also released following exposure to radiation. However, the finding of elevated levels of histamine is not universal; in one patient with urticaria,³⁴ the serum histamine level was reported to be normal and in another, no histamine could be detected in the dermal perfusate after the patient was exposed to a dose of radiation sufficient to produce urticaria.¹⁷ It is possible that the assays used in these studies were insufficiently sensitive or, alternatively, that histamine is not the predominant mediator in all cases. The latter possibility is supported by the finding that histamine depletion by compound 48/80 and intra-dermal administration of antihistamines were successful in blocking the urticarial response in some, but not all, cases of solar urticaria.^{17,23,28}

11.7.3. Other Factors

Local injection of epinephrine prevented a reaction in patients with urticaria who were subsequently exposed to radiation.^{17,28} Presumably this effect is caused by vascular constriction and unresponsiveness. The reaction has also been delayed or prevented by occlusion of the local arterial supply.^{11,14,15,17} Occlusion is most readily accomplished by placing a sphygmomanometer cuff on the arm and irradiating the forearm; as little as 1 min of occlusion during the exposure to radiation is sufficient to prevent the reaction.¹⁴ This observation has been put forward as evidence that creation of the antigen is a photodynamic process requiring oxygen and that the low oxygen tension in the tissues that result from arterial occlusion inhibits the formation of the antigen. However, other factors must be considered: (1) A cuffed arm is not bloodless; (2) blood contains other components apart from oxygen which may be involved in the urticarial reaction; and (3) arterial occlusion undoubtedly also alters vascular responsiveness. It is of interest that arterial occlusion blocked the delayed papular response to radiation in a patient with actinic reticuloid,²⁰ a condition quite unrelated to solar urticaria. Therefore, the effect may be nonspecific.

11.8. Conclusions

Solar urticaria is a clearly defined entity with remarkably uniform clinical features, but individual cases appear to have quite differing photobiologic and immunologic features. The photobiologic characteristics of solar urticaria require further examination, particularly with high-intensity broadband sources of radiation. Since a broad range of wavelengths appears to be responsible for the disease, little can be gained by detailed monochromatic studies. Large exposure doses of broadband radiation should be used to study solar urticaria so that even minimal reactivity can be detected, thus providing more useful information. Further serum transfer studies are required, but these are restricted by the hazards of performing the examination in humans and the expense of conducting them in primates, a necessary requirement since human IgE binds only to mast cells of closely related species. However, despite these difficulties, transfer studies may permit investigation of the nature of the antigen and further characterization of the antibody. For example, absorption of serum by cellular components that have been irradiated *in vitro* may provide a clue to the photoproduct responsible for the interaction with the serum factor that gives a positive transfer reaction. The organ culture technique for studying human skin may also provide a means for *in vitro* study of solar urticaria.

Finally, although an immunologic mechanism is suggested by the findings in some cases of solar urticaria, in many cases there is now no evidence for involvement of the immune system except, perhaps, the name urticaria.

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Chapter 12

Photoallergy

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12.1. Introduction

Photoallergy is a state of specifically altered reactivity towards an exogenous chemical in which photons play some role. The usual manifestation is an eczematous rash that occurs after the skin is exposed to both an offending chemical and electromagnetic radiation. In many conditions classified as photoallergic, the evidence for an immune mechanism is suggestive, not conclusive. Immunologic reactivity in photoallergy is thought to be directed toward an antigen that arises as the result of a reaction(s) initiated by the absorption of radiation by (1) the chemical or a complex containing the chemical, (2) a biotransformation product of the chemical, or (3) a host molecule altered by the chemical.

Chemicals may produce photosensitivity directly by absorbing radiation or indirectly by producing a disease state which is expressed as photosensitivity. The latter conditions include drug-induced lupus erythematosus, porphyria cutanea tarda produced by alcohol and estrogens, and pellagra caused by anti-tuberculous therapy. These conditions are not relevant to the present discussion, and the term *chemical photosensitivity* is used here only to refer to conditions in which direct absorption of the appropriate activating wavelengths of radiation by the exogenous chemical plays a role.

Chemical photosensitivity can be due to either photoallergy or phototoxicity. The pathogenesis of chemical phototoxicity does not involve the immune system. In addition, its clinical and histologic characteristics are quite different from photoallergy. However, considerable confusion has arisen in the clinical literature because of a failure to distinguish clearly between photoallergy and phototoxicity. For example, in clinical reports, drugs and other chemicals are frequently described as having caused a photoallergic response when little or no evidence is presented to indicate that the immune system is involved. In many instances, these reactions are probably phototoxic in nature and it is preferable to label

them chemical photosensitivity, a general term which does not imply a mechanism for the reaction, until the pathogenesis is established.

Photoallergy is probably a cell-mediated immune response in which specifically sensitized lymphocytes interact with the photoallergen. In this type of reaction, the specific state of altered reactivity can be passively transferred by lymphocytes from a sensitized host. The photoallergic response is usually manifest as eczema in the skin at the site of exposure, and histologic examination reveals spongiosis of the epidermis and a mononuclear infiltrate in the dermis. Thus, photoallergy has features in common with ordinary allergic contact hypersensitivity. The first contact with the photoallergen does not result in an immediate response because an induction phase of 1 to 2 wk is required. A response may be seen at the sensitization site after the induction phase or, alternatively, not until 12 to 48 hr after a subsequent exposure to the photoallergen. Furthermore, photoallergy is restricted to certain susceptible individuals; therefore, only a proportion of exposed individuals are affected regardless of the exposure doses of chemical and radiation.

Phototoxic and photoallergic reactions are similar in that both involve absorption of photons by a chemical or a complex containing the chemical, but in other respects phototoxic reactions are quite different from photoallergic reactions. The immune system is not involved in mediating a phototoxic response, and damage to cells and tissues is quite nonspecific. The main clinical features of phototoxicity are erythema followed by pigmentation, although, rarely, urticaria-type reactions may occur. Large doses of a chemical and/or radiation can result in edema, blistering, and desquamation. The changes seen on histologic examination of the skin vary depending on the site of the photobiologic reaction. For example, epidermal damage similar to that seen following exposure to UVB radiation may result, or, alternatively, the epidermis may remain intact and the principle feature may be a cellular infiltrate in the dermis or subcutaneous tissue. A phototoxic reaction follows the first exposure to a chemical and radiation and usually appears within hours. Subsequent exposures at previously untreated sites mimic the time course and appearance of the first exposure. Finally, a phototoxic chemical will produce a similar response in all exposed individuals, provided the dose of chemical and radiation are adequate.

Photoallergy and phototoxicity are related in that certain chemicals act as both photoallergens and phototoxins. The response produced in a given situation probably depends on host factors, the dose of chemical, and the dose of radiation. Following *in vivo* exposure, many photoallergens can also produce phototoxicity. This dual activity of photosensitizing compounds obviously can lead to confusion in interpreting the nature of clinical responses and *in vivo* test procedures such as the photopatch test. To establish that a reaction is photoallergic in nature, it is necessary to provide evidence for involvement of the immune system and the presence of a specific state of altered reactivity.

Photoallergy also overlaps with ordinary allergic contact hypersensitivity, since some photoallergens can induce both reactions. When a suspected photoallergen is found to produce contact hypersensitivity (also frequently termed contact allergy), careful testing is necessary to demonstrate that it also produces a separate reaction that requires a specific interaction with radiation. Photoexacerbation of allergic contact hypersensitivity, which is simply enhancement of contact hypersensitivity by exposure to UV radiation, can also cause confusion. In this response, absorption of radiation by the chemical is probably not involved, and thus it is distinct from photoallergy.

12.2. History

Photoallergy was first suggested as a pathogenesis for photosensitivity by Epstein in 1939.¹ Reports of sulfanilamide photosensitivity had begun to appear, and he tested the photosensitizing capacity of this agent in normal volunteers. After the site of intradermal injection of sulfanilamide was exposed to broadband sources of radiation, erythema appeared in all subjects within 1 to 24 hr and lasted several days. The erythema response was followed by pigmentation. This reaction required minimally erythemogenic doses of radiation, and its occurrence in all tested subjects, plus the clinical features, are consistent with a phototoxic response. Ten days later, two of the six subjects developed a spontaneous reaction with urticaria-type lesions at the site of the original injections. Repeated testing of these two subjects resulted in similar urticaria-type lesions 12 to 24 hr after challenge with sulfanilamide plus radiation. Histologic examination of these lesions was not performed, so it is not possible to further interpret the response, but it is likely that this reaction was photoallergic in nature.

Photosensitivity to sulfonamides was a common problem during the period when these agents were used extensively as antimicrobial agents. Then, photosensitivity was observed following both topical and systemic administration. Sulfonamides are not used often today, but related compounds, the thiazide diuretics, are used widely and frequently produce photosensitivity. These agents cause phototoxicity,² but it is not clear whether they can also produce a photoallergic reaction.

The next major epidemic of photoallergic reactions occurred in the early 1960s and was caused by tetrachlorosalicylanilide (TCSA), an antibacterial agent in soaps.² In England, thousands of cases were observed in the short period of time that the soap products containing this agent were marketed. Other halogenated salicylanilides and some closely related compounds continue to be used as germicidal agents in toiletries. The photosensitizing capacity of these compounds varies a great deal, and the frequency and geographic distribution of reports of salicylanilide photosensitivity have largely depended on the

marketing practices of the companies involved. The topical germicidal agents used in toiletry preparations today, such as the chlorocarbanilides, appear to have a very low potential for inducing photosensitivity. In recent years, interest in photoallergy has centered on various components of sunscreens such as glyceryl para-aminobenzoic acid, benzophenones, and 6-methylcoumarin. Musk ambrette, which is widely used in perfumes and as a food additive, has also attracted interest as a photoallergen recently.

12.3. Photoallergic Chemicals

In the last 40 years, many chemicals and drugs have been reported to cause photoallergy. However, in most cases, the data supporting the immunologic nature of reactions has been incomplete. For example, a compound suspected as the etiologic agent for a photosensitivity reaction was tested by the photopatch technique, and a positive response was seen at the site of application of chemical and exposure to radiation. Alternatively, a patient with photosensitivity had a positive response to a photopatch test upon screening with known photosensitizers. In both instances, a positive photopatch test result was the primary evidence that the compound was responsible for a photoallergic reaction, but as discussed later in this chapter, photopatch testing may not always distinguish reliably between phototoxicity and photoallergy.

Ideally, the following criteria, which are discussed in following sections, should be satisfied before a chemical is labeled as a photoallergen:

1. Clinical: eczema at the site of exposure to chemical and radiation.
2. Histologic: spongiosis of the epidermis and a mononuclear cell infiltrate in the dermis.
3. Testing: positive photopatch test.
4. Experimental:
 - a. Induction of the photoallergic response to the chemical in man or an experimental animal.
 - b. Positive responses to the chemical plus radiation in in vitro tests that correlate with delayed hypersensitivity.
 - c. Transfer of hypersensitivity to naive animals.

Tetrachlorosalicylanilide is the only compound that satisfies all these requirements, but a few other compounds satisfy many of them. The compounds listed in Table 12.1 meet some of these criteria and can be considered as probable photoallergens.

Table 12.1
Contact Photoallergens

3,3',4',5-Tetrachlorosalicylanilide (TCSA)
3,4',5-Tribromosalicylanilide (TBS)
4',5-Dibromosalicylanilide (DBS)
Bithionol
Hexachlorophene
3,4,4'-Trichlorocarbanilide (TCC)
Dichlorophene
4-Chloro-2-hydroxybenzoic acid N- <i>n</i> -butylamide (Jadit)
Sulfanilamide
<i>p</i> -Aminobenzoic acid esters
<i>o</i> -Hydroxybenzophenones
6-Methylcoumarin
Chlorpromazine
Musk ambrette

12.4. Incidence

The incidence of photoallergy in the population is unknown, but may be higher than one would expect from the scattered case reports in the literature. Many cases of photoallergy may be incorrectly diagnosed as eczematous polymorphic light eruption (PMLE) or photosensitive eczema because photoallergy is not suspected. Alternatively, the diagnosis of photoallergy may be considered, but a search for the photosensitizer is unsuccessful. Deficient knowledge about what photosensitizing chemicals are in the environment, the lack of a simple method for establishing the diagnosis of photoallergy, and a poor understanding of the pathogenesis of the condition are major reasons why photoallergy is a gray zone in photomedicine.

12.5. Clinical Features

Photoallergic reactions can occur at any age and in either sex. Males are more commonly affected, but this may well be due to greater exposure to the allergens and sunlight rather than to any intrinsic difference in susceptibility. The patient usually presents with a rash, which he or she attributes to sunlight exposure because the rash is restricted to the exposed areas of the body. The initial eruption frequently occurs a few days after a long exposure to sunlight. Subsequently, an eruption may appear or become more severe within hours of exposure to the eliciting wavelengths of radiation. Since a photoallergic response results from an

interaction between radiation and a chemical, the actual distribution of the eruption is determined by the dose and site of exposure to each agent. The eruption, of course, is largely restricted to areas exposed to nonionizing radiation.

The vast majority of photoallergic reactions are eczematous in character. The predominant feature in the early stages of the eruption is vesication, which may progress to bullae, with scaling, crusting, and excoriations as variable features. In the chronic stage, lichenification with thickened plaques are the main features. Urticular, papular, and lichenoid eruptions have also been reported as photoallergic reactions, but these are unusual responses described in isolated case reports. Erythema alone, in the absence of any epidermal change, is probably always a manifestation of phototoxicity rather than photoallergy.

The first step in the diagnosis of photoallergy is to establish that the eruption is eczematous in nature. Clinical examination alone is not adequate and histologic confirmation is always essential. By these means, phototoxicity can usually be excluded as a possible diagnosis, leaving in the differential diagnosis all other eczematous conditions of exposed areas of the body. Contact dermatitis from airborne substances can be very difficult to distinguish from photoallergy because the distribution of the eruption is usually very similar. Lack of a close relationship to sun exposure, the occurrence of the eruption in certain geographic locations, and clustering of cases are the usual clues leading to a correct diagnosis of airborne contact dermatitis. Sun-exacerbated atopic eczema should be suspected in a female patient with a history of atopic eczema and involvement of the flexural surfaces of skin. Photosensitive eczema and the eczematous form of PMLE are two diagnoses of exclusion that should be considered only after an exhaustive search for a photoallergen. It is quite possible that these conditions are manifestations of photoallergy to chemicals that have not yet been identified as photoallergens.

The next stage in the diagnosis is to identify the photoallergen. Some clues to its identity may be obtained from a careful history, but usually the patient must be tested to all known photosensitizers. The photopatch test is the usual method of testing for reactivity to photosensitizing chemicals.

A photoallergic response will continue as long as the subject remains exposed to the chemical, or a cross-reacting chemical, and radiation. Thus, the eruption may persist for only about a week following a single contact with the offending agent, such as application of a sunscreen containing a photoallergen, prior to a single sun exposure. Alternatively, the eruption may be of long duration if a substance such as a photosensitizing soap is used daily. The treatment of a photoallergic response is to remove the allergen and/or reduce exposure to radiation; the eruption should then subside and leave no residual damage. Pigmentation is usually not a sequela of a photoallergic reaction, and its presence points towards phototoxicity. Occasionally, the eruption will remain after the offending agent has been removed, and this situation suggests either continued contact with a crossreacting chemical or the development of a persistent light reactor state; the latter condition is discussed in Section 12.5.3.

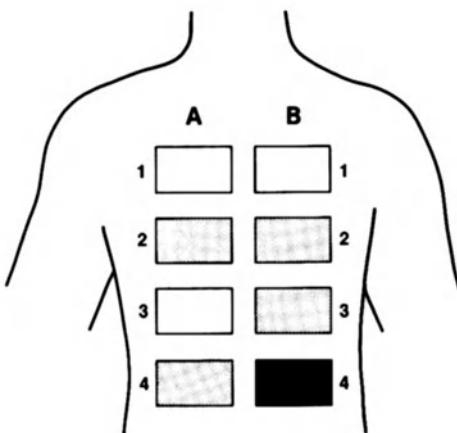


Figure 12.1. Photopatch testing. Chemicals are applied to sites A1 to A4 and B1 to B4. Sites B1 to B4 are exposed to UVA radiation 24 hr later. At 48 hr, positive responses are interpreted as follows: A2 and B2 are ordinary contact allergy; B3 is photocontact allergy; A4 and B4 are ordinary contact allergy and photocontact allergy.

12.5.1. Photopatch Testing

This is the most widely used approach to the investigation of photoallergy. There is no universally accepted, standard technique for the procedure, but the approaches used by most clinical photobiologists are fairly similar. Known photosensitizers are applied in duplicate to symmetrical small areas of the back, and all sites are covered by an opaque material. After 24 hr, one set of the applied chemicals is exposed to UVA radiation, and 24 to 48 hr later, the sites are examined for reactions. Erythema at any site, with or without edema and vesiculation, is recorded as a positive response and is often graded on a 1+ to 4+ scale depending on the severity of the reaction. Positive responses of equal intensity at both the nonirradiated and irradiated sites are interpreted as consistent with ordinary contact allergy (Fig. 12.1). A positive response at an irradiated site or, in the presence of ordinary contact allergy, an enhanced response at the irradiated site is interpreted as consistent with photocontact allergy.

There are several limitations to the use and interpretation of the photopatch test, and various modifications have been introduced to circumvent these problems. The radiation sources vary, but most investigators use a source with the main emission spectrum in the UVA range. The UVA waveband has been selected because the action spectra of photoallergic reactions to most chemicals are included in this range of wavelengths. However, the action spectra of some reactions, such as those to sulfanilamide³ and diphenhydramine,⁴ probably involve UVB radiation. To avoid this problem, a solar simulator which emits both UVA and UVB radiation is sometimes used. However, such a combined source introduces problems of dosimetry because UVB radiation is much more erythemogenic than UVA radiation. When a UVA radiation source is used for photopatch testing, the exposure dose is usually $1 \times 10^5 \text{ J/m}^2$, a dose arbitrarily selected because it is below the minimal erythema dose for most individuals and is presumably a "sufficient dose to elicit a photoallergic response." A suberythema dose of radiation

is usually administered with a solar simulator, and, depending on the filters used and the sensitivity of the subject to UVB radiation, the exposure dose of UVA radiation can vary widely under these conditions. Thus, it seems more logical to use different patch test sites for each waveband of radiation. In addition, since the action spectra of some photosensitizers lie in the visible range, patch test sites should also be exposed to this waveband. Furthermore, to maximize the chance of detecting a photoallergic response in any given individual, the doses of radiation should be dictated by a determination of the minimal erythema dose to each waveband in that individual. The use of doses just below the erythema threshold offers the best chance for detecting photosensitivity reactions.

The selection of doses of photosensitizers to be used for challenge tests has been determined by trial and error in patients and volunteers. Ideally, the dose is below the toxic or irritating dose of the chemical alone and below the phototoxic dose in the case of chemicals with that property. However, the phototoxic dose is difficult to determine because there is considerable interpersonal variation in phototoxic responses and most studies have employed only small numbers of people for testing. Indeed, phototoxicity has occurred in many individuals with the doses of some photosensitizers that are used in photopatch testing.⁵ This raises the question of how to interpret a positive photopatch test result. Presuming that only one reaction occurs at the test site, visual distinction between phototoxicity and photoallergy in a small area is fraught with difficulty, particularly if the accepted end-point is erythema. Some investigators are confident in making this visual distinction, but others also use a histologic evaluation of test sites. Unfortunately, we lack both careful comparative studies of the histologic features of phototoxic versus photoallergic responses in small test sites and detailed studies of the time course of these responses at a microscopic level, which could provide a guide to the best time for biopsy. An obvious complicating factor in the visual and histologic evaluation of a positive response is the simultaneous occurrence of phototoxicity and photoallergy. It is unlikely that either method of evaluation could detect photoallergy under such circumstances.

Certain photoallergens, at times, can probably produce ordinary contact allergy, and the photopatch test is designed to differentiate between these two reactions. If the nonirradiated site is kept covered with an opaque material for the full 48 hr and there is a positive response, it can be interpreted as signifying ordinary contact allergy to the chemical. However, in the early literature, reactions that appeared several days after the site was uncovered were interpreted as being consistent with ordinary contact allergy with an unusual time course for the challenge response. It is more likely, as has been demonstrated,⁶ that these reactions represented activation of the photoallergen by small doses of radiation in the ambient lighting when the subject had his or her back uncovered. Photoexacerbation of ordinary contact allergy is also a problem. For example, chromium sensitivity can be exacerbated by exposure to UV radiation.⁷ This effect, presumably due to UVB radiation, probably is not related to photoallergy, but could be caused by a phototoxic action of the hapten or be a nonspecific

effect of radiation on an inflammatory response. However, it raises the question of how to interpret the results when simultaneous patch and photopatch tests are positive. This situation requires careful evaluation of the clinical picture in the patient in terms of photosensitivity versus contact allergy, as well as further studies of the photosensitizing potential of the chemical in animal models.

In summary, the technique of photopatch testing obviously has value in the investigation of a patient with photosensitivity and in the study of photoallergy, but the methodology used and the results obtained should be evaluated critically. The sensitivity and accuracy of the test can be maximized by careful attention to dosimetry of both the chemical and radiation, by use of multiple wavebands of radiation, and by visual and histological evaluation of reactions in all cases. All these parameters require further study to define the conditions of the test system.

12.5.2. Contact and Systemic Photoallergy

It is often assumed that contact photoallergy (epicutaneous introduction of the chemical) and so-called systemic photoallergy (oral or parenteral introduction) have a similar immune pathogenesis. However, although there is some evidence to support an immune pathogenesis for contact photoallergy, there is virtually none to support such a pathogenesis for skin eruptions described as systemic photoallergy.

In reports of photosensitivity reactions following systemic administration of drugs and other chemicals, two pieces of evidence are usually provided to support an immune pathogenesis: The presence of eczema and a positive photopatch test to the suspected photoallergen. Neither piece of evidence is strong support for involvement of the immune system. Eczema can result from stimuli such as dryness of the skin without apparent involvement of the immune system. Furthermore, in systemic photosensitivity, the strongest reaction to a chemical may be in the dermis rather than the epidermis; therefore, distinguishing between phototoxicity and systemic photoallergy on photopatch testing may be even more difficult than distinguishing contact photoallergy from phototoxicity.

Many cases of systemic photosensitivity are probably phototoxic in nature, but there is no theoretical reason why a chemical cannot give rise to a photoallergic reaction as easily when it reaches the skin via the bloodstream as when it is introduced epicutaneously. However, further investigations of thiazides and other drugs are required to demonstrate that systemic photoallergy exists and that it has an immune pathogenesis.

12.5.3. Persistent Light Reactor

The majority of patients with a photoallergic-type reaction to a chemical cease to show any abnormal response to radiation when exposure to the offending chemical is stopped. However, some patients continue to display photosensi-

tivity, even when there is apparently no further exposure to the chemical or other crossreacting chemicals. This clinical condition, termed the *persistent light reactor state*, was first recognized by Wilkinson⁸ as a sequela of the epidemic of salicylanilide photoallergy. Since then it has been recognized in patients sensitized by a number of compounds, and it is likely that the condition can be a complication of any photoallergic reaction. The frequency of the reaction may vary with different photoallergens, but it is estimated that 25 to 30% of patients sensitized to salicylanilides developed a persistent reaction.^{9,10} The condition can also occur as a complication of systemic photosensitivity, as we have observed it in a patient with thiazide-induced photosensitivity.¹¹ The disorder has several features: Marked photosensitivity that is manifest as eczema, a positive photopatch test to the original photoallergen, and a lowered minimal erythemal dose with UVB radiation and possibly also with longer wavelengths, even though the action spectrum for the original photoallergic reaction may have been confined to the UVA range.

Patients with a persistent light reaction do not appear to have any special features associated with the initial episode of photosensitization, although there is a suggestion that prolonged contact with the photoallergen may be a factor. Photosensitivity is often so marked that it is impossible for affected patients to tolerate even minimal sun exposure. The eruption is a chronic eczematous dermatitis, often with marked lichenification, and it is restricted to light-exposed areas of the body. Photopatch tests reveal a strongly positive reaction to a photoallergen and any similar cross-reacting compounds. Phototesting of normal skin in the absence of the chemicals reveals a strikingly lowered minimal erythemal dose of UVB radiation, with an acute eczematous response at the test sites on both clinical and histologic examination.^{12,13} Responses to UVA radiation can also be abnormal; a low minimal erythemal dose of UVA radiation and eczematous reactions have been reported.^{14,15} Sensitivity to visible radiation is suggested because the reaction can be triggered by room lighting in some patients, but this may be caused by the small amount of UV radiation emitted by some fluorescent bulbs. Ramsay¹⁵ examined the response to visible radiation in four patients with persistent light reaction and did not find abnormal responses, but it is possible that the challenge doses in this study were too low. The prognosis in this condition can be very bleak because the photosensitivity may persist for 20 yr or more.

The mechanism of the persistent light reactor state is unknown but has given rise to much speculation. One hypothesis, which is very difficult to prove or disprove, is that these patients remain in contact with the original photoallergen or a related cross-reacting chemical. However, this does not explain why these patients are sensitive to UVB radiation since, at least with salicylanilides, the activating wavelengths are in the UVA range. An alternative hypothesis is that the photoallergen remains bound in the skin for long periods of time. Proponents of this theory have studied the time course for clearance of TCSA from skin. In

human volunteers, TCSA could still be detected in skin 6 months after the initial exposure.¹⁶ In guinea pig skin, evidence of persistence of TCSA or a photoproduct was found by fluorescence 3 wk after the initial application of chemical.¹⁷ Of course, it is somewhat difficult to imagine that a compound would persist in skin for 20 yr or that sensitization by a photoallergen in a hair cream would produce a persistent generalized state of photosensitivity as indicated by abnormal phototest responses on buttock skin.¹⁵ Apart from these considerations, antigen persistence does not explain the increased sensitivity to UVB radiation or the negative responses to UVA radiation on noninvolved skin in persistent light reactors.¹⁸ Another hypothesis to explain this condition is that the antigenicity of some normal constituent of the body is altered during the photoallergic reaction. According to this theory, the initial photochemical insult produces a photoproduct that is antigenic and induces an immune response. Subsequent exposure to radiation triggers the immune response by formation of the same or a closely related "normal" photoproduct from an endogenous substance. Evidence for photochemical alteration of the carrier protein in an *in vitro* system involving TCSA has been obtained, so this mechanism is at least theoretically possible.¹⁹

In one study of persistent light reaction precipitated by bithionol, it was found that the photosensitivity in one patient could be suppressed by treatment with sunlight and 8-methoxysoralen.¹⁴ The minimal erythema dose of UVB radiation in the patient returned to the normal range after treatment, and the authors attributed this response to the protective effect of pigmentation and thickening of the stratum corneum. The observation could have been carried further by performing a photopatch test with bithionol in a treated area; a positive reaction to bithionol would have provided some indirect evidence that the persistent light reaction was a separate and distinct response to the original photoallergy.

The persistent light reaction is one of the most intriguing aspects of photoallergy and obviously requires much more study. It is unfortunate that this condition has not been reported in animals, since an animal model could provide the best approach to investigating the pathogenesis of the reaction.

12.6. Pathogenesis

Photoallergy involves the absorption of photons by a chromophore, photochemical interactions leading to the formation of an antigen, and the induction of an immune response. Unfortunately, our knowledge of some of these steps is deficient, but this is probably due to a lack of investigation rather than any intrinsic mystery associated with this immune response. Furthermore, the exact mechanism may vary with different chemicals.

12.6.1. Experimental Models

Contact photoallergy has been induced in both man and experimental animals. The procedures used always involve application of the photoallergen and exposure to a source of UVA radiation. Additional measures, such as the use of irritants and immunological adjuvants, have been included in most protocols to increase the degree and frequency of sensitization. In man, contact photoallergy was induced to TCSA, tribromosalicylanilide (TBS), bithionol, hexachlorophene, 3,4,4'-trichlorocarbanilide (TCC), and 4,4'-dichloro-3-(trifluoro)carbanilide.²⁰ A 10% concentration of each of these chemicals in petrolatum was applied in a thin layer to 2-inch squares of skin which were then exposed to solar-simulated radiation (UVB, UVA, and visible spectrum from a xenon lamp). This procedure was repeated four times at the same site at 48-hr intervals. Prior to the first treatment, but not to the subsequent ones, the skin site was tape-stripped to the glistening layer. Testing for a photoallergic response was performed 2 wk later on normal skin. Sites treated with 1% concentration of the test substances in petrolatum were irradiated with UVA and visible radiation from the xenon source. These sites were examined 24, 48, and 96 hr later and compared to the reaction at sites exposed to the chemical but not irradiated. The eczematous nature of the reaction was confirmed histologically, but the number of subjects photosensitized to each chemical was not clearly stated. The main evidence for the role of immunologic mechanisms in these instances of "photoallergic" contact dermatitis was by analogy with ordinary allergic contact dermatitis. However, this study demonstrates that photosensitizing chemicals can produce an eczematous response in human skin. In a similar study, a group of normal volunteers were sensitized to 6-methylcoumarin and related compounds, and an eczematous response was elicited on challenge with the compounds plus exposure to radiation.²¹

In vivo studies in humans have obvious ethical, financial, and time constraints which limit their usefulness in the investigation of photoallergy. Furthermore, although comparative studies are few, it is not certain that studies in humans have any advantage over the use of animal models.

Guinea pigs have been most often used in the study of photoallergic responses. Induction of experimental photoallergy in guinea pigs was first reported in 1957 by Schwarz and Speck, who used sulfanilamide.²² In general, the induction procedure involves application of the compound to the depilated nuchal region, exposure to UVA radiation, and treatment by some other technique that facilitates sensitization. The techniques used are exposure to UVB radiation,²³ topical application of 20% sodium lauryl sulfate,²⁴ skin-stripping with cellophane tape,²⁵ and intradermal injection of Freund's complete adjuvant.²⁶ The advantages and disadvantages of these techniques have been recently reviewed.²⁷ UVB radiation has been used most frequently, although the other three techniques produce higher incidences of sensitization. The mechanism by which these various agents

facilitate sensitization is unknown, but it is clear that at least in the guinea pig, exposure to UVA radiation plus the chemical is not sufficient to produce photoallergy. Cripps and Enta¹⁸ were unable to produce photoallergy in the guinea pig with a chemical plus UVA radiation, even when the induction phase was continued for 16 wk. However, UVB and UVA radiation in combination induced sensitization within a week. It is possible that the role played by techniques such as tape-stripping and detergent application is simply to reduce the barrier function of skin. The sensitization step has to be repeated several times and is usually performed 3 to 10 times over a 7- to 10-day period.

The elicitation test is performed 14 to 21 days after the last sensitization treatment. Two or three concentrations of the test compound are applied to duplicate sites on the depilated lumbar area of the back. One set of sites is exposed to UVA radiation. The concentration required for elicitation depends upon the compound and the induction technique. For example, when the skin-stripping technique is used, 5% musk ambrette is required, but when Freund's adjuvant is used, only 0.1% musk ambrette elicits a positive response.²⁶ Reactions are usually scored 24 to 72 hr after the challenge treatment. Erythema, or erythema with edema, are recorded as positive responses.

Many of the studies of photoallergy in guinea pigs have been directed principally towards developing a suitable model for induction and elicitation of the response. The questions raised by this emphasis are whether photoallergens are inherently weak sensitizers, or alternatively, whether the guinea pig is unsuitable for these studies. Recently, Maguire and Kaidbey have induced photoallergy to TCSA and other compounds in BALB/c and other strains of mice.²⁸ Weak sensitivity was achieved by applying the chemicals and subsequently exposing the mice to UVA and UVB radiation. However, sensitivity was markedly enhanced by pretreating the animals with cyclophosphamide. We have confirmed this finding and found that when mice are pretreated with cyclophosphamide, UVB radiation is unnecessary for induction of the immune response.²⁹ We now use the following protocol: 150 mg/kg cyclophosphamide is injected intraperitoneally. On the third and fourth days after injection, 1% TCSA is applied to the shaved backs of the animals and they are exposed to 10^5 J/m² UVA radiation. The ears are shielded during the exposure to radiation. Seven days later, 1% TCSA is applied to the ears and the animals are exposed to 10^5 J/m² UVA radiation. Finally, 24 hr later, the thickness of the ears is measured to assess the photoallergic response. Cyclophosphamide presumably inhibits suppression of the response, an action observed with this agent in contact hypersensitivity.

12.6.2. Photobiologic Aspects

This aspect of photoallergy has been studied for only a few compounds. This section summarizes our present understanding.

12.6.2.1. Wavelength Dependence

In experimental animals and in man, the cutaneous response to most photoallergens is elicited by UVA radiation. The long wavelength limit of this range is set by the end of the absorption spectra of the photoallergic compounds. Although there is no basic photochemical reason why the absorption spectra of photoallergic compound. That is, photoallergic responses were obtained in the 360- to 390-nm visible radiation. Further testing may identify such compounds, since a number of photosensitizers are activated by visible radiation. The shorter wavelength limit may be determined by the competitive absorption of photons below 300 nm by other skin components.

Cripps and Enta¹⁸ determined action spectra for the response to three halogenated salicylanilides. Their action spectra closely followed the absorption spectra of the photoallergic compounds at wavelengths longer than 320 nm. For instance, the maxima of action spectra in two patients tested with TCSA was 360 nm, which is the maximum in the absorption spectrum of the anionic form of TCSA (Fig. 12.2). This correlation is important because it indicates that the molecular environment of TCSA for elicitation of a photoallergic reaction has a pH greater than 8. In one patient tested by Cripps and Enta, the action spectrum for reaction to bithionol was 30 nm longer than the absorption spectrum of this compound. That is, photoallergic responses were obtained in the 360- to 390-nm range where bithionol does not absorb. The lack of correspondence between the action spectrum and the absorption spectrum can be attributed to several factors such as (1) *in vivo* binding of the compound to cellular components which shift the absorption spectrum, (2) metabolism to produce a new chromophore which

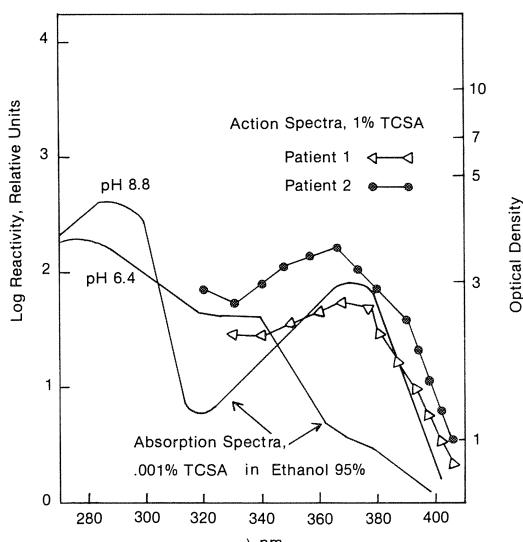


Figure 12.2. Action spectra for TCSA photopatch tests in two patients and absorption spectra of TCSA in 95% ethanol at pH 6.4 and 8.8. Modified from Cripps DJ, Enta T: Absorption and action spectra studies on bithionol and halogenated salicylanilide photosensitivity. *Br J Dermatol* 82:230-242, 1970.

is the actual photosensitizer, and (3) a shift to longer wavelengths due to the influence of the optical properties of skin. Action spectra established by Freeman and Knox³⁰ for responses to four compounds indicated that radiation below the UVA range may also contribute to photoallergic reactions. However, in guinea pigs photosensitized to musk ambrette, the action spectrum for the photoallergic response corresponded to the absorption spectrum (322 to 406 nm).³¹ Examination of both the morphology of the response and the histologic changes in the skin showed that, at wavelengths below 310 nm, there was a normal delayed erythema response and above 310 nm, there was a photoallergic response.

Therefore, at least for the salicylanilides and musk ambrette, the action spectrum for photoallergy appears to fall mainly within the UVA waveband. However, Blum³ found that the elicitation of sulfanilamide sensitivity in one subject could be prevented by a glass filter which absorbed all wavelengths shorter than 320 nm from the mercury arc source. In an *in vitro* study, we found that sulfanilamide phototoxicity for lymphoid cells was induced by exposure to UVB radiation, but not UVA radiation.³² Bearing in mind that skin optics may alter the action spectrum *in vivo*, our findings tend to support those of Blum. There is also a report of a patient with photosensitivity to diphenhydramine which could be elicited by exposure to UVB radiation, but not UVA radiation.⁴ As indicated by these isolated reports, the general assumption that chemical photosensitivity is induced by exposure to UVA radiation alone may not be valid in all cases.

12.6.2.2. Chemical Concentration and Radiation Dose Dependence

In both experimental and clinical situations, the doses of chemical and radiation are critical to a photoallergic response at two stages: induction and elicitation. The frequency of induction and elicitation of photoallergic responses might be expected to increase as either the amount of chemical or the dose of radiation is increased, since the number of photons actually absorbed by the chemical depends on the number of photons available and the concentration of the chemical. Of course, a saturation level may be reached, and it is conceivable that the development of phototoxicity may suppress the immune response.

Systematic studies on the concentrations of sensitizer or the dose of radiation required for induction of contact photoallergy have been limited. Typically, the photosensitizer is applied as a 1 to 10% solution in ethanol or acetone or it is suspended as a solid in petrolatum. Photoallergy to sulfanilamide was induced in guinea pigs with injections of a 1% solution.²² The dose of UVA radiation is generally 1 to 3×10^5 J/m².

In general, the concentrations of photoallergic compounds used to elicit photoallergy are lower than those used to induce photoallergy. For example, Morikawa et al.³³ used 2% solutions of halogenated salicylanilides for induction of photoallergy and 0.01, 0.1, and 1% solutions for elicitation. In contrast, the

concentrations of musk ambrette needed to elicit photoallergy (5 and 7%) were nearly the same as that used in the induction (10%).³¹ The relationship between the concentration of the allergen and the frequency of elicitation of a positive response was tested by Osmundsen⁵ in patients with TBS photoallergy. The number of patients who responded to photopatch tests using 0.0001, 0.001, 0.01, and 0.1% concentrations of TBS, dibromosalicylanilide (DBS), and monobromosalicylanilide (MBS) in this study is shown in Figure 12.3.

The dose-dependence of the intensity of the response to an elicitation dose of a photoallergen was shown with musk ambrette.²⁶ Elicitation with 1% musk ambrette produced erythema with marked edema in 60% of animals, erythema with edema in 30%, and erythema alone in 10%. In comparison, a concentration of 0.1% resulted in a 10%, 30%, and 60% incidence of these graded responses. The dependence of the elicitation response on the dose of UVA radiation has not been reported.

The concentration of different chemicals required to elicit the same frequency of photoallergic response depends upon the compound. Several factors may be involved, including (1) differences in absorption spectra between compounds, (2) differences in penetration of chemicals to the appropriate layer of the skin, (3) differences in the degree of photosensitization produced by the induction procedure, and (4) differences in the photosensitizing potential of the compound.

12.6.2.3. Photo-cross-reactivity

Positive responses in sensitized patients or experimental animals to challenge with UVA radiation plus a compound which is not the primary photosensitizer are termed photo-cross-reactivity. This phenomenon is often observed in photo-

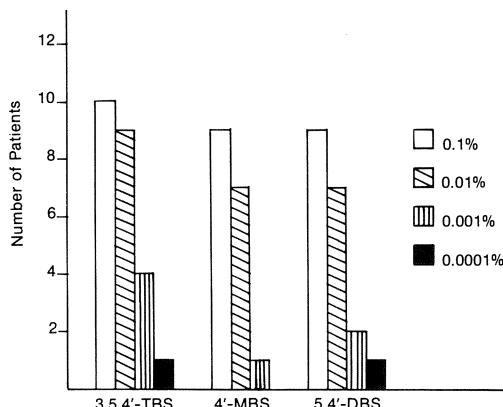


Figure 12.3. Results of photopatch tests in 10 TBS-sensitive patients tested with TBS, DBS and MBS at four concentrations in methyl ethyl ketone. From Osmundsen TE: Photopatch testing. *Trans St John's Hosp Derm Soc* 55:160-173, 1969.

sensitized patients. Studies of groups of photosensitized individuals have shown that most respond to more than one test compound in a photopatch test. For example, in the study by Epstein et al.¹² of the 25 patients with contact photoallergy who were tested with up to eleven compounds, 23 had positive reactions to more than one substance. Contact allergy to the test materials was also observed in some patients. Similarly, when contact photoallergy was experimentally induced in volunteers,²⁰ all TBS-photosensitized subjects were also contact-sensitized to 4',5-DBS and to 4'-MBS, and all TCSA-photosensitized subjects were also contact-sensitized to trichlorosalicylanilide (TCS) and dichlorosalicylanilide (DCS).

In guinea pigs, photo-cross-reactivity to TBS, MBS, bithionol, and hexachlorophene was detected in animals sensitized to TCSA.³⁴ In a larger study, Morikawa et al.³³ sensitized groups of guinea pigs to six brominated halogenated salicylanilides. Animals sensitized with each compound showed photo-cross-reactivity to at least one other compound in the series and usually to two or more. Animals sensitized to TCSA photo-cross-reacted with six other chlorinated salicylanilides, with five brominated salicylanilides, and with salicylanilide. No photo-cross-reactivity was observed with compounds which are not closely related structurally, such as bithionol, fentichlor, hexachlorophene, salicylic acid derivatives, and aniline derivatives. The mechanism for photo-cross-reactivity will be discussed in this chapter.

12.6.2.4. Phototoxicity

The possibility that most photoallergens are also phototoxic has attracted much interest because this property offers the potential for a rapid screening test for photoallergic chemicals. Both *in vivo* and *in vitro* systems have been used to assess the phototoxic properties of photoallergens. The phototoxicity of photoallergic compounds has been tested in experimental animals.³³ At a 1% concentration, 3,5-DBS, TCSA, 3,5-DCS, and 3-MBS were phototoxic in combination with 1×10^5 J/m² radiation in guinea pigs. In rabbits, under the same experimental conditions, the same compounds as well as 5-monochlorosalicylanilide (5-MCS), phenothiazine, chlorpromazine, bithionol, and fentichlor were phototoxic. Sulfanilamide, TBS, 4',5-DBS, MBS compounds, TCS, 3',5'-DBS, and 3'- and 4'-MCS were not phototoxic to either animal under the conditions of that study. This study illustrated important limitations of an *in vivo* test system for phototoxicity: (1) It is difficult and time-consuming to conduct adequate dose-response studies *in vivo* and (2) there are marked species differences in the response to a phototoxic insult. *In vitro* studies could possibly avoid these problems. The targets for detecting phototoxic chemicals *in vitro* have included mammalian cell lines, red blood cells, microorganisms, and biochemical reactions. The

main problem with these tests has been that of obtaining false-negative results; chemicals that are clearly photosensitizers in humans are often not phototoxic in the assays. We have evaluated human lymphoid cells as a target for detection of phototoxic chemicals in combination with broadband sources of UVA and UVB radiation.³² No false-negative results were obtained with the 12 known photosensitizers tested, so that this test may have value as a predictive assay.

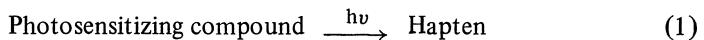
12.6.3. Photochemical Aspects

The role of radiation in the induction of photoallergy is in the formation of the antigen. Photons could be absorbed either by the photosensitizing compound or by another skin component that would then react with the photosensitizing compound. The action spectra for photoallergic responses reflect the absorption spectra of the photosensitizing compounds, and this strongly suggests that the compounds are the chromophore.

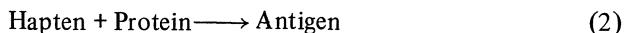
There are at least three molecular mechanisms by which an antigen could be formed following absorption of photons by the photosensitizing compound:

12.6.3.1 Formation of a Photoproduct Which Acts as a Contact Allergen

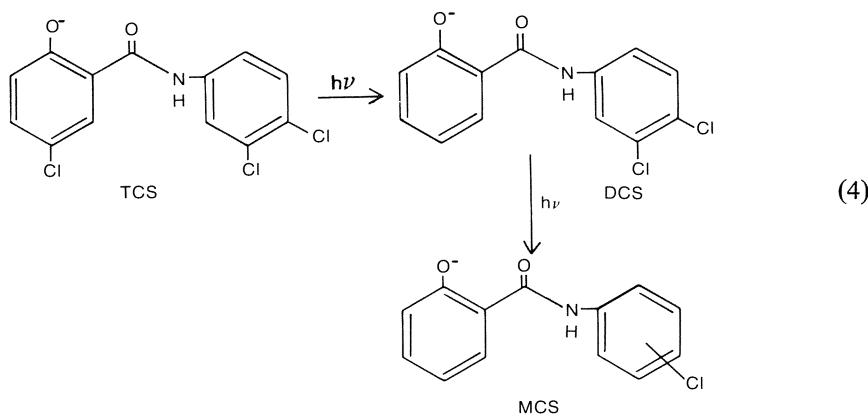
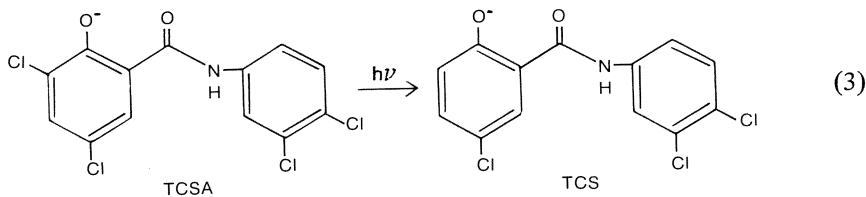
Upon absorption of a photon, the photosensitizing compound is chemically altered to form another stable compound, the hapten (Eq. 1).



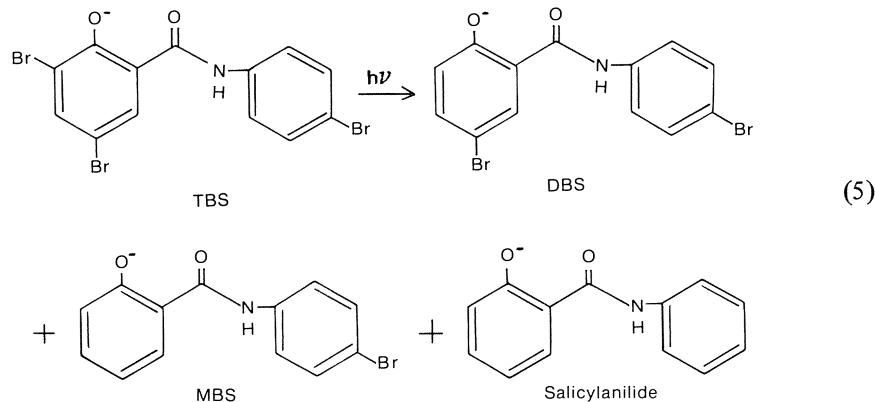
The hapten then combines with protein without further absorption of radiation (Eq. 2) and the hapten–protein conjugate is the antigen as in ordinary contact allergy.



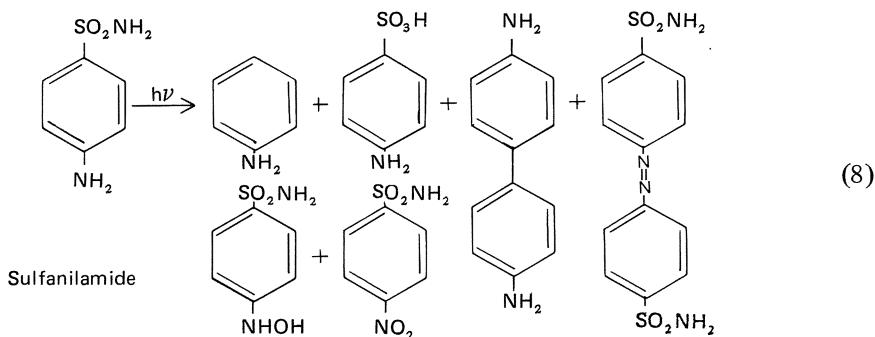
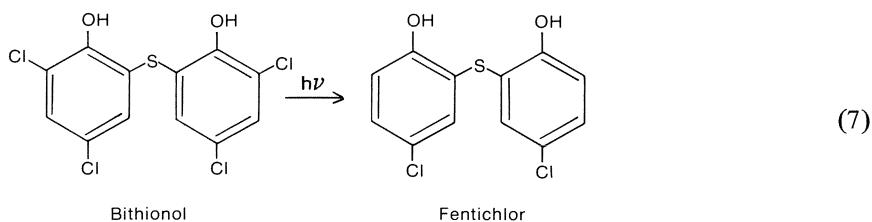
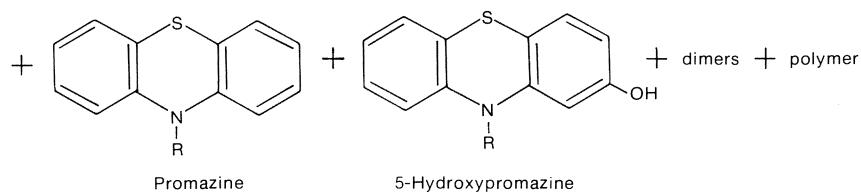
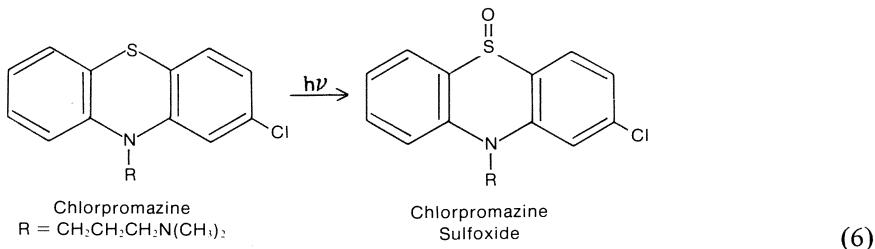
Experiments to test this hypothesis have been designed to answer the following question: Do photochemical reactions of photoallergic compounds generate contact sensitizers? Photochemical studies in vitro have identified stable photoproducts from several photoallergic compounds. Irradiation of TCSA in aqueous solution caused initial loss of the chlorine next to the hydroxy anion (Eq. 3)³⁵ and subsequent loss of the other chlorines (Eq. 4).³⁶ The photochemistry occurs with the anionic form of TCSA^{19,36} instead of the protonated form. This result is consistent with the action spectra of Cripps and Enta.¹⁸

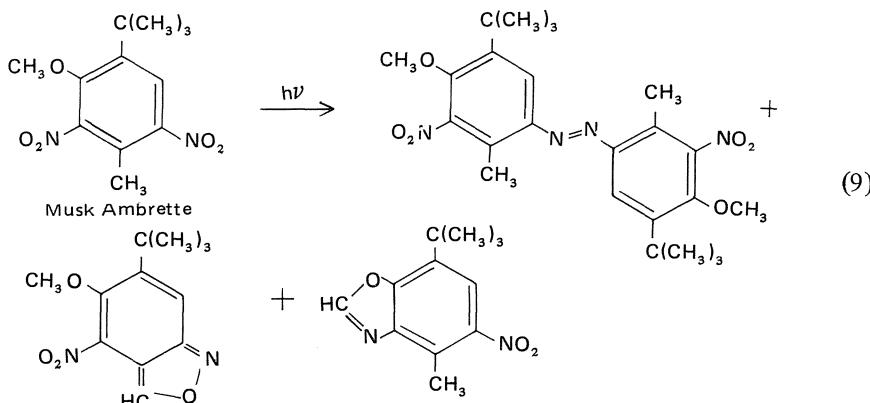


Irradiation of TBS also results in dehalogenated photoproducts (Eq. 5).^{33–35}



Photoproducts have been identified following the in vitro photochemical alteration of chlorpromazine (Eq. 6),³⁷⁻³⁹ bithionol (Eq. 7),³³ sulfanilamide (Eq. 8),⁴⁰ and musk ambrette (Eq. 9).⁴¹





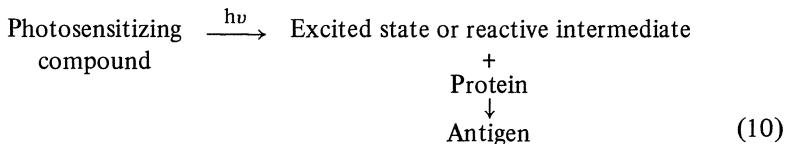
The potential of several of these photoproducts to act as contact sensitizers has been evaluated. Willis and Kligman²⁰ tested ten volunteers, who were previously photosensitized to either TBS or TCSA, with the pure photoproducts of these compounds. Contact allergy to the photoproducts was found in all cases, and the intensity of the reaction increased as the number of halogens on the photoproduct decreased. In ten other volunteers, it was determined that contact allergy could be induced to the photoproducts of TBS and that the sensitizing potential increased in the order TBS < DBS < MBS. These results and positive simple patch tests to MBS in patients contact photosensitized to TBS^{5,42} are consistent with the photochemical generation of a contact allergen from photoallergic compounds. However, results from much more detailed experiments in guinea pigs showed a more complex picture.³³ Photosensitization by TCSA produced significantly higher degrees of contact allergy and contact photoallergy than did its photoproducts, TCS and DCS. Simple contact sensitization by TCSA, TCS, and DCS gave results with no apparent pattern. The photoproducts from TBS were more potent inducers of contact allergy and contact photoallergy than TBS itself. However, the photoproduct of bithionol, fentichlor, was a more potent inducer of contact photoallergy than bithionol but neither compound induced contact allergy.

In summary, although studies in humans support the hypothesis that photoproducts of photosensitizing compounds can act as contact allergens, data obtained in animals suggest that this one mechanism cannot explain all cases. Furthermore, there is no evidence that photoproducts of photosensitizers can covalently bond to protein in the dark.

12.6.3.2. Photochemical Conjugation of the Photosensitizing Compound to Protein

According to this proposed mechanism, the photosensitizing compound in an excited state or a short-lived intermediate derived from the compound reacts

with a carrier protein to form an antigen (Eq. 10). In contrast to the previous mechanism, a stable photoproduct is not involved.



In support of this mechanism, *in vitro* photochemical experiments have indicated that reactive intermediates (free radicals) are formed upon irradiation of several photoallergic compounds. The structures of some of these free radicals have been established. These include the radicals formed from halogenated salicylanilides,³⁶ chlorpromazine,³⁹ and *p*-aminobenzoic acid.⁴³ In general, halogenated compounds photoreact by halogen–carbon cleavage to yield a pair of radicals. Other electron-rich compounds such as chlorpromazine, sulfanilamide, and *p*-aminobenzoic acid produce hydrated electrons upon photoexcitation. Amino-substituted compounds photoreact by losing the amino group and generating radicals. In all these systems, the radicals produced can react in several ways, some of which may lead to covalent binding between a protein and the altered photoallergic compound. The detailed molecular mechanisms have not been determined. It has been established that covalent bonding between proteins and photoallergic compounds can result from photoexcitation. Such photon-initiated bonding has been demonstrated for TCSA,^{19,44} chlorpromazine,⁴⁵ and 4-chloro-2-hydroxybenzoic acid *N*-*n*-butylamide,⁴⁶ which has the trade name of Jadir.® Covalent bonding of TCSA and albumin following exposure to radiation occurred only after noncovalent bonding in the dark.⁴⁷ In addition, TCSA could bond to more than one site on the albumin molecule.

In summary, *in vitro* photochemical studies lend support to the hypothesis that a direct photochemical reaction between the photosensitizing compound and protein could be the mechanism of antigen formation in some cases of contact photoallergy. The available data suggest that both this and the previous mechanism may apply in different cases.

12.6.3.3. Photochemical Alteration of a Protein

According to this proposed mechanism, the photosensitizing compound absorbs radiation and then photochemically alters a protein which then becomes the antigen. There is little evidence to support this possibility; however, this could be due to a lack of investigation. One study has demonstrated that TCSA *in vitro* can photosensitize the oxidation of histidine in human serum albumin,¹⁹ but the antigenicity of this altered protein has not been tested *in vivo*.

12.6.4. Immunologic Aspects

The evidence that photoallergy involves an immunologic pathogenesis has, until recently, been largely circumstantial. However, the development of a model for photoallergy in inbred strains of mice has provided the opportunity for exploring the immunologic characteristics of this response. Photoallergy to TCSA has been transferred to naive animals by injection of lymphoid cells from sensitized syngeneic mice.²⁸ Furthermore, it was found that elimination of B lymphocytes from the donor cells did not affect the response in the recipient mice. This observation suggests that T lymphocytes might mediate photoallergy, but more direct evidence is required. A failure to transfer sensitivity to naive animals with lymphoid cells depleted of T lymphocytes would be the best evidence for involvement of that cell population in the immune response.

The mouse model for photoallergy will provide an opportunity to study many aspects of the pathogenesis of this immune response. For example, we have found that pretreatment of animals with a large dose of UVB radiation, suppresses the development of photoallergy.²⁹ Thus, photoallergy shares this characteristic of ordinary contact hypersensitivity. The nature of the antigens involved in photoallergy, systemic photosensitivity due to chemicals, and the persistent light reaction are other exciting areas for further study in this model.

A question that must be raised is whether findings in an experimental model for photoallergy in the mouse can be applied to clinical photoallergy in humans. Demonstration of a similar response in another species would provide some indirect evidence that photoallergy is similar in all species. Guinea pigs would be another useful model for such studies because (1) much is known about their immune system, (2) the photobiologic characteristics of its skin are more similar to humans than are those of mouse skin, and (3) inbred strains are available. However, little work has been done in this direction. There is one report⁴⁸ of successful transfer of photoallergy to TCSA in the Hartley strain of guinea pigs, but only one naive animal was sensitized. A larger study, preferably using inbred animals, is required to confirm this finding. The nature of the immune response in the guinea pig has also received some attention. Herman and Sams⁴⁴ found that a TCSA-protein conjugate inhibited the migration of peritoneal exudate cells obtained from guinea pigs sensitized to TCSA. This *in vitro* assay is accepted as a test for antigen-stimulated production of migration-inhibition factor by sensitized T lymphocytes. The hapten, TCSA, was conjugated to guinea pig albumin, and this antigen inhibited the migration of cells by an average of 90%, as compared to 10% with TCSA alone and 5% with the protein. The migration of cells from nonsensitized animals was not inhibited. However, the specificity of the response was not examined by testing with other antigens.

Demonstration that photoallergy in humans is a cell-mediated immune response, as it appears to be in other species, will require the development of in

vitro assays for photoallergy. Jung and co-workers⁴⁶ have done some work in this direction. They used an antigen-stimulated lymphocyte transformation assay to investigate the immune nature of photoallergy. This in vitro assay is generally accepted as a correlate of delayed hypersensitivity. The chemical tested in their study was 4-chloro-2-hydroxybenzoic acid *N*-n-butylamide, a compound related to the salicylanilides. Photosensitivity to this chemical has been reported in countries where it has been marketed as an antifungal preparation. A hapten-albumin complex was used as the antigen to test for transformation of lymphocytes obtained from photosensitive individuals. Transformation was stimulated by the complex but not by the chemical or protein alone. The major drawback of the study was that transformation was assessed qualitatively on the basis of the morphology of the cells rather than by the quantitative method of measuring incorporation of a radioactive tracer into DNA of dividing cells. There have been considerable advances in techniques for in vitro investigation of delayed hypersensitivity since this study was reported, and it is somewhat surprising that further investigations have not been forthcoming to support the findings.

The role of antibodies in photoallergy was investigated by Herman and Sams,⁴⁹ but their findings were entirely negative. Sera from sensitized human subjects and guinea pigs did not give precipitation lines when tested against an epidermal protein-salicylanilide mixture using the Ouchterlony immunodiffusion technique. Furthermore, sections of skin treated with TCSA and exposed to radiation did not show evidence of antibody binding when tested with serum from sensitized animals and humans using the indirect immunofluorescence technique. Biopsy specimens of skin from humans and animals were also tested for deposition of immunoglobulin and complement by the indirect immunofluorescence technique, but again the results were negative. The authors concluded that salicylanilide photoallergy did not involve formation of antibodies to the photoallergen. In delayed hypersensitivity reactions such as contact allergy, antibodies are probably not involved in the immune response, but they may be produced. Thus, the negative findings in these studies do not support or detract from the concept of photoallergy being an immune response.

12.7. Conclusions

There are a number of unanswered questions in the area of photoallergy and the techniques are available to provide the answers. Photoallergy appears to be a cell-mediated immune response that can be transferred by lymphoid cells, but the characteristics of the cells responsible for this transfer require further examination. This and other characteristics of the immune response can now be studied in the mouse model that has been developed. Parallel studies in an inbred strain of guinea pigs would also be very useful. The pathogenesis of systemic photo-

sensitivity due to chemicals and the persistent light reaction is another area that can be explored in animal models. Questions also remain as to the photochemical alterations involved in photoallergy and these are probably best approached by combined in vitro and in vivo techniques. Testing of the antigenicity in vivo of photoproducts generated in vitro would help determine the mechanism responsible for formation of the antigen.

Because new photoallergens will continue to be introduced into the environment and many existing photoallergens probably have not been identified, there is a need for a reliable screening procedure to detect these substances. Ideally, this would involve a simple in vitro test combined with animal testing of any suspect compounds. At present, in vitro testing for phototoxicity provides an indirect approach to screening chemicals for photoallergenicity, but a specific in vitro test for photoallergens would be preferable.

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Chapter 13

Autoimmune Disease

Warwick L. Morison

13.1. Introduction

Various diseases have been classified as being autoimmune because responses to self-antigens appear to be involved in their pathogenesis. Numerous theories have been put forward to explain the phenomenon of autoimmunity, but with our current understanding, the two that are most attractive are based on the formation of new and cross-reactive antigens or a loss of normal immune regulation. According to the theory of cross-reactive antigens, an exogenous agent may modify a normal body constituent and increase its antigenicity, or alternatively, antigenic determinants of the agent itself may crossreact with normal host antigens. The concept of a loss of normal immune regulation leading to the development of autoimmunity has become more attractive with increased understanding of the intricacies of immune function. For example, a loss of normal suppressor T cell function could lead to overactivity of other T cells or of the humoral immune system, with resultant abnormal cell- or antibody-mediated immune responses.

Autoimmunity is of interest in photoimmunology because several diseases that appear to have an autoimmune pathogenesis can be induced or exacerbated by exposure to nonionizing radiation. Lupus erythematosus (LE) is the best example of such a disease, but in addition, the blistering skin diseases pemphigus and bullous pemphigoid also are affected by nonionizing radiation. There are isolated reports of other autoimmune diseases, such as dermatomyositis and rheumatoid arthritis, having been exacerbated following exposure to sunlight, but there is no real evidence to suggest that nonionizing radiation is involved in the pathogenesis of these conditions.

It is quite possible that nonionizing radiation could induce an autoimmune response by way of either of the two mechanisms that have been outlined. For example, exposure to UV radiation could photochemically alter a normal cellular constituent so that it is more antigenic and able to stimulate the formation of

antibodies. These antibodies could then react with the photoproduct on subsequent exposure to radiation or crossreact with the original cell constituent to produce an immune response. There is evidence that this may occur with DNA, and it is quite conceivable that exposure to UV radiation could alter other molecules in a similar manner. In addition, UV radiation is toxic to lymphocytes, and alterations of lymphocyte function have been observed following *in vivo* exposure to UVB radiation. Thus, it is possible that UV radiation could selectively affect a lymphocyte subpopulation involved in the regulation of a specific immune response. Of course, this would be most likely to occur in a subject whose lymphocytes, because of some genetic or environmental influence, had a greater than normal susceptibility to the toxic effects of radiation.

An alternate possibility is that the photosensitivity seen in autoimmune disease represents a nonspecific response to inflammation. According to this hypothesis, any trauma that produces an inflammatory response should have a similar effect. At present, there is little evidence to suggest that UV radiation can induce such a nonspecific effect, but it is fair to add that most investigation has been directed toward exploring the specific, rather than the nonspecific, effects of radiation in autoimmune disease.

13.2. Lupus Erythematosus

Lupus erythematosus is a chronic inflammatory disease that can affect multiple organs including the skin. Although the fundamental cause of LE is unknown, the many immunologic abnormalities that are characteristic of the disorder strongly suggest an immune pathogenesis. Nonionizing radiation also can play a role in the pathogenesis, as is evidenced by photosensitivity, which is a common clinical feature, and by the fact that the disease is often precipitated and exacerbated by exposure to sunlight. However, despite much investigation, it is not clear whether nonionizing radiation acts by way of a specific effect on immune function or as a nonspecific toxin that produces an inflammatory response.

13.2.1. Clinical Findings

Various types of LE are distinguished on the basis of clinical and laboratory findings, but it is likely that they all represent a continuum. Discoid LE is at one end of the spectrum with clinical disease confined to the skin and few or no associated systemic immunologic disturbances. The skin lesions usually are clearly defined plaques, which develop slowly and persist for a long period. Systemic LE in its most florid form is at the other end of the disease spectrum and is manifest as a multisystem disorder with marked immunologic abnormalities. The skin is involved in 60 to 70% of patients and the morphology varies from typical discoid

lesions to acute erythemas. Joint involvement is the most common systemic manifestation, but the kidneys, heart, lungs, and nervous system are also frequently affected.

Photosensitivity is a common feature of LE, and it occurs in various forms. Skin eruptions occur mainly on sun-exposed areas of the body, with the face being affected most frequently. Thus, sun exposure probably plays a role in producing the eruption in most cases. However, only in a small percentage of patients is there a history of a particular exposure to sunlight being responsible for initiation of the eruption. Exacerbation of an existing eruption by exposure to sunlight is much more common and occurs in the majority of patients with discoid LE and about 30% of patients with systemic LE. In addition, in systemic LE, the involvement of other organs can be triggered or exacerbated by excessive exposure to sunlight; while this is usually associated with cutaneous photosensitivity, it can occur in the absence of skin involvement.

13.2.2. Laboratory Abnormalities

The most frequently used screening test for LE is the indirect immunofluorescence test for serum anti-nuclear antibodies. This test is positive in almost all cases of systemic LE, but in most patients with discoid LE, the test is negative or positive only at a low antibody titer. Similarly, antibodies to native DNA are present in many patients with systemic LE, but not in patients with discoid LE. Recently, an antibody (anti-Ro) to a cytoplasmic protein has been identified in some patients with LE, and it has been found that the majority of these patients are photosensitive.¹ The pathophysiologic significance of this observation is unclear, but it is an important finding because many of these patients have a negative anti-nuclear antibody test and yet have the clinical picture of systemic LE.

13.2.3. Histology and Immunopathology of the Skin

Histologic examination of skin is a useful means of diagnosing LE. Changes vary with the morphology of the eruption, but consistent features are liquefaction degeneration of the basal cell layer and a patchy infiltrate of lymphocytes in the dermis, particularly around appendages. Deposits of fibrin within the connective tissue of the dermis are frequently seen in systemic LE, but are uncommon in discoid LE.

Deposits of immunoglobulin and complement are present in the basement membrane zone of the skin lesions. Detection of the deposits by direct immunofluorescence constitutes what is called a positive lupus band test. The immunoglobulin most commonly found in the deposits is IgG, but trace amounts of IgM may be present also. Components of both the classical and alternate complement

pathways may be present. The lupus band test is negative in the uninvolved skin of patients with discoid LE. In systemic LE, the test is positive in sun-exposed normal skin in almost all cases and in nonexposed normal skin in about 50% of patients.

13.2.4. Photobiologic Studies

The cutaneous responses to UV radiation are often abnormal in patients with LE, as manifested by the development of clinical lesions, prolongation of the duration of the delayed erythema response, and, possibly, increased sensitivity to UVB radiation.²⁻⁶ In these studies, patients were phototested with single or multiple exposures of small areas of skin to various sources and doses of radiation; no systemic exacerbation of symptoms was observed as a result of the phototesting. Lesions that were clinically and histologically consistent with LE were produced in some patients who had a history of photosensitivity, but patients who did not have such a history did not develop lesions. These findings applied to patients with systemic LE and discoid LE. In one study in which suberythemogenic doses of UV radiation were used,² the development of lesions was delayed for 7 to 42 days after exposure, whereas in other studies,^{3,4} the erythema response probably evolved into characteristic lesions. All induced lesions regressed over 2 to 12 wk in the absence of specific treatment. In the lesions of two patients, a positive lupus band test with deposits of IgG and IgM was found at both 7 and 24 wk, whereas this test had been negative 2 wk after exposure to radiation.³ From the information provided in this report, it is not possible to determine whether skin lesions preceded the deposition of immunoglobulin. The action spectrum for production of lesions in LE has not been clearly defined, but from the limited observations made, it appears that wavelengths less than 320 nm are very active. The effects of wavebands in the UVA and visible region were examined to a limited extent with negative results,⁴ but it is possible that the exposure doses were inadequate to evoke a reaction.

The delayed erythema response to UVB radiation was found to be of abnormally long duration in some patients and has been observed to persist as long as 10 wk without histologic evidence of LE in the skin.⁴ This abnormality is most common in patients with a history of photosensitivity,³ but it can be seen in the absence of such a history.⁴ There is also the suggestion that, in patients with LE and a history of photosensitivity, the minimal erythema dose of UVB radiation is at the lower limit of normal,³ but this observation has not been confirmed. The interpretation of the reports of phototesting in patients with LE is somewhat difficult because many of the patients were being treated with corticosteroids and/or antimalarial drugs at the time of the examination. These treatments may suppress the development of lesions in susceptible individuals, possibly explaining the failure of UVB radiation to produce lesions in all patients with a

history of photosensitivity. Also unexplained is the relationship between the deposits of immunoglobulin at the level of the basement membrane, the appearance of clinical lesions, and the development of histologic changes.

13.2.5. Pathogenesis

The cause of LE is unknown, but the development of overt disease appears to involve genetic predisposition, environmental factors, and hormonal influences. These interactions result in multiple disturbances of immune functions, some of which are probably associated only with the disease while others may be responsible for its manifestations.

A genetic influence is suggested by a high concordance for systemic LE in monozygotic twins, a family history of the disease in about 5% of the patients, and a high incidence of serum autoantibodies in first-degree relatives. Furthermore, a Mendelian dominant pattern of inheritance is seen in some animal models of LE. An increased frequency of certain histocompatible antigen genotypes in patients with LE has been reported in several studies, but usually this association has been weak; other studies have reported no correlation.

Lupus erythematosus, particularly in its systemic form, predominantly affects women, and the peak incidence is in those women of child-bearing age. Furthermore, exacerbations of the disease are common in pregnancy and can be induced by administration of oral contraceptives. These observations suggest a role for sex hormones as modifiers of the disease process. This concept is supported by observations in animals with an LE syndrome. Male NZB/W mice develop lupus nephritis later than females. However, in males castrated at birth, the age at onset is like that in females, and in females given male hormones, the age at onset assumes the male pattern.

Various environmental influences have been implicated in the development of LE, including drugs, viruses, trauma, and exposure to UV radiation. Drugs can produce a lupus-type syndrome, apparently as a pharmacologic effect in most affected individuals (for instance, those taking hydralazine and procainamide), but also as an idiosyncratic effect with many other agents. Viral involvement in the pathogenesis of LE has been suggested by many studies, but a causative agent has not been isolated and the role of viruses in induction of the disease remains obscure. Trauma to the skin commonly triggers lesions in patients with discoid LE, but it does not appear to be an important factor in systemic LE.

13.2.5.1. Immunologic Aspects

The function of the immune system is grossly disturbed in LE and this is most evident in systemic LE. The most obvious evidence of this disturbance is the presence of a whole array of autoantibodies directed against nuclear and

cytoplasmic macromolecules; against cells such as lymphocytes, erythrocytes, and platelets; and against other proteins such as IgG and thyroglobulin. Some of these antibodies are involved in the disease process, particularly antibodies to DNA and other nuclear constituents. Tissue damage appears to be mediated by the formation of immune complexes and the subsequent fixation and activation of complement.

Evidence suggests that the overactivity of the humoral immune system is secondary to a quantitative and qualitative deficiency of suppressor T cell activity and that this deficiency correlates with the activity of the disease. T cells may be directly involved in the production of tissue damage, but this is not clearly established. K cells and macrophages may also show a functional impairment in systemic LE.

The reasons for the deficient suppressor T cell activity and for the selective overactivity of B lymphocytes towards endogenous, but not exogenous, antigens are unknown.

13.2.5.2. Photoimmunologic Aspects

Exposure of DNA to UV radiation results in the formation of photoproducts known as UV-DNA. UV-DNA is antigenic; in animals, it has been shown to induce the formation of antibodies that react specifically with UV-DNA and native DNA (nDNA).⁷ DNA is a weak antigen and chemically pure nDNA and denatured DNA (dDNA) do not induce the formation of antibody in animals. Plescia, Braun, and Palczuk⁸ found that a complex of denatured calf thymus DNA and methylated bovine serum albumin could produce in rabbits an antiserum which reacted with dDNA, but not with nDNA. Using this technique, Levine et al.⁷ showed that when the dDNA was exposed to UVC radiation before it was combined with methylated bovine serum albumin, the antiserum produced in rabbits exhibited much greater reactivity with irradiated nDNA or dDNA than with unirradiated DNA in the complement fixation reaction. They cited three findings which suggested that antigenic determinants in UV-DNA were thymine photo-products: First, the serologic activity of the antiserum was greatest with DNA that had a high thymine content; second, binding of the antiserum was decreased if, after exposure to 270-nm radiation, DNA was then exposed to 235-nm radiation, which breaks pyrimidine dimers; and third, irradiated thymine oligonucleotides inhibited the reaction of the antibody with UV-DNA. These findings were confirmed and extended in a study in which the reaction between anti-UV-DNA and dDNA was inhibited by thymine nucleotides, but not by thymine dimers.⁹ It has also been found that nDNA exposed to UVC radiation is immunogenic in rabbits.¹⁰ The activity of antiserum produced in this way was inhibited by irradiated nucleotides and nucleosides containing thymine, but not cytosine.¹¹ Re-

sults of a recent study¹² suggest that, although the immunologically active lesions in UV-DNA include thymine photoproducts, conformational changes in the structure of DNA are also important.

UV-DNA, which is produced *in vivo* following exposure of the skin to UV radiation, has been identified by an immunofluorescent antibody technique in mouse¹³ and human¹⁴ skin. In hairless mice, UV-DNA can be detected in epidermal cells for 24 hr following exposure to UVC radiation, but it is not detected at 48 hr.¹⁵ Serum obtained from mice for up to 15 hr after exposure to radiation inhibited the activity of antibodies directed against UV-DNA,¹⁶ which suggests that the photoproduct is released from skin into the circulation. The action spectrum for formation of UV-DNA in mouse skin has been examined and includes the waveband from 254 to 320 nm.¹⁷ One study failed to demonstrate formation of UV-DNA with a radiation source that had an emission spectrum of 290 to 400 nm,¹⁵ but the UVA waveband has not been investigated further.

UV-DNA is produced upon exposure of the skin to UVR; there also is evidence that it is immunogenic *in situ* and can stimulate the production of antibodies. When mice with high titers of anti-UV-DNA were exposed to UVC radiation, immunoglobulin and complement were deposited in the exposed skin in a pattern similar to that seen in patients with LE,¹⁸ suggesting that circulating antibody to UV-DNA may play a role in the pathogenesis of the skin lesions in LE. According to this theory, UV-DNA formed as a result of exposure to radiation is released from the epidermis and immunizes the host, who then forms antibodies to UV-DNA. On subsequent exposure to radiation, the anti-UV-DNA reacts with the photoproduct in the skin, fixes complement, and gives rise to an inflammatory response. This theory was partly supported by the finding that the incidence of positive antinuclear antibody tests is higher in Swiss mice exposed to UVC radiation daily for 8 wk than in control mice exposed only to daylight.¹⁹ However, it is interesting that the antinuclear antibodies found in the exposed animals reacted with both irradiated and nonirradiated substrates. A similar study using NZB/W mice, which spontaneously develop a disease resembling systemic LE, failed to show any differences between mice exposed to UVC radiation and those kept in the dark in terms of mortality, appearance of proteinuria, or the development of a positive antinuclear antibody test.²⁰ Mice are not the best model for investigating the pathogenesis of skin lesions in LE because there is no clinical evidence of skin involvement in the animals that have been studied. However, it is interesting that deposits of immunoglobulin and complement occur spontaneously at the basement membrane zone in NZB/W mice.

Antibodies to UV-DNA have been found in the sera of patients with systemic LE.¹⁰ In many patients with LE, these antibodies show immunologic specificity for UV-DNA and are distinct from antibodies that react with nDNA.²¹ However, in the latter study, there was a poor correlation between the presence of anti-

bodies to UV-DNA and a history of photosensitivity. Unfortunately, the criteria for deciding whether a patient was photosensitive were not defined. The presence of antibodies to UV-DNA should be compared in patients who develop lesions upon exposure to UV radiation and those who do not develop such lesions.

Some other effects of UVR in LE have been investigated. Defective repair of DNA following exposure to UVC radiation was observed in lymphocytes from patients with systemic LE in one study,²² but a similar study, using fibroblasts obtained from skin biopsies of patients with LE, found normal repair synthesis.²³ A photoactive, low-molecular-weight, chromosome-damaging agent was found in the serum of patients with systemic LE.²⁴ Lymphocytes from patients with systemic LE were found to be more sensitive to 360- to 400 nm radiation, as judged by trypan blue dye exclusion, than those obtained from normal subjects. Furthermore, addition of the chromosome-damaging agent to normal lymphocytes before they were exposed to this waveband of radiation produced an increase in the number of chromosomal aberrations and a decrease in the viability of the cells as compared to cells exposed only to radiation. The authors suggested that the photoactivated agent was an unstable hydroperoxide released from the lymphocytes of patients with systemic LE as a result of exposure to UVA radiation and that it played a role in the photosensitivity of such patients. No details were provided as to whether the patients examined were indeed photosensitive. This intriguing finding requires further study and confirmation.

13.2.6. Conclusions

The evidence for involvement of nonionizing radiation in the pathogenesis of LE is that the disease can be triggered and exacerbated by exposure to sunlight and that it is possible to reproduce the skin lesions by deliberate exposure to UV radiation. The question that arises is whether these changes are due to a specific alteration of immune function induced by the exposure to radiation. Clearly, UV radiation can induce specific changes in the antigenicity of DNA, but so far it has not been possible to relate these changes to the development of the disease process. Antibodies to UV-DNA could well be just another expression of the disease and have nothing to do with the pathogenesis of LE. Of course, if that possibility should prove to be true, it would not exclude a role for UV radiation in the pathogenesis of the disease, since radiation may act on some other fundamental control mechanism such as suppressor T lymphocytes. The main effect of UV radiation in LE is on the development of skin lesions, but unfortunately, studies of the actions of UV radiation have been limited because there is no animal model available for studying the skin changes in LE. However, the effect of UV radiation on the evolution of skin lesions in humans has not been fully explored and that might be a useful first step in increasing our knowledge of the action of UV radiation in this disease.

13.3. Pemphigus and Bullous Pemphigoid

These two blistering disorders of unknown etiology are characterized by immunologic reactions in the skin that appear to be responsible for the development of lesions. Bullous pemphigoid and certain types of pemphigus can be induced or exacerbated by exposure to sunlight, and lesions have been reproduced on normal skin of patients by exposing the skin to artificial sources of radiation. However, as is the case with LE, the specificity of the effect of nonionizing radiation in these diseases has not been established.

13.3.1. Pemphigus

Several clinical varieties of this disease are recognized but all are characterized by the formation of intraepidermal blisters. Circulating antibodies to the intercellular substance of the epidermis frequently are found in the serum, and direct immunofluorescence examination of involved skin, and at times uninvolved skin, reveals a deposition of immunoglobulin and complement between the cells of the epidermis. IgG antibodies are very important in the pathogenesis, but IgA and IgM antibodies can also be detected in the skin. Whole serum or the IgG fraction of serum obtained from patients with pemphigus produces intraepidermal blistering and intercellular immunofluorescence when it is injected into the lip mucosa of monkeys; such serum also produces the histologic changes of the disease when it is incubated with normal human skin in vitro.

A localized form of pemphigus called *pemphigus erythematosus* can be induced or exacerbated by exposure to sunlight. Lesions can be reproduced on apparently normal skin of patients by exposing the skin to erythemogenic doses of UV radiation,^{25,26} whether or not the patients have a history of sensitivity to sunlight. Evidence of a developing lesion can be detected within 24 hr of exposure because the skin of these patients is more fragile than that of normal persons, and typical blisters are seen after 2 to 8 days. There is some evidence that induction of lesions by UV radiation can be prevented by prior medication with chloroquine but not by moderate doses of prednisone.²⁶ Other forms of trauma, including application of a chemical irritant, tape occlusion for 24 to 48 hr, and exposure to a heat lamp, were not found to induce lesions.²⁶

Another form of pemphigus called *pemphigus vulgaris*, which is not associated clinically with sunlight sensitivity, can be reproduced at a microscopic level in the apparently normal skin of patients by exposure to UV radiation.²⁷ Deposits of IgG and, in some cases, of complement were seen 5 hr after exposure. Twenty-four hours after exposure, these deposits were much more noticeable and were present in all patients. However, histologic evidence of formation of an intraepidermal blister was detected only at 24 hr postexposure. The morphologic appearance of the skin, in terms of erythema or clinical lesions, was not mentioned

in that report. Subsequent to phototesting, corticosteroids were used in two patients, and after their disease had been cleared, they were retested for their response to UV radiation, presumably while still on treatment. Under these circumstances, UV radiation did not induce microscopic evidence of disease or deposition of immunoreactants, which suggests that prednisone can block the response.

The influence of UV radiation on the development of lesions consistent with pemphigus has also been studied *in vitro* using an organ culture system.²⁸ Normal human skin was established in culture with and without the addition of serum obtained from a patient with pemphigus vulgaris. The skin was irradiated either *in vivo* prior to excision or *in vitro*, using a broadband source of UV radiation. The dose of radiation used was equal to that found to produce lesions *in vivo* in patients with pemphigus vulgaris. Intraepidermal blistering occurred in the skin in organ culture and was associated with deposition of IgG, but these alterations were not enhanced by the exposure to UV radiation *in vitro* or *in vivo*. One possible explanation for this negative finding could be a lack of involvement of complement in the organ culture system. Pemphigus antibodies do not fix complement *in vitro*, but there is good evidence that activation of complement *in vivo* is associated with the development of lesions.

These studies have demonstrated that exposure to UV radiation can reproduce lesions in patients with pemphigus and that deposition of immunoglobulin and complement is associated with this event. However, except for some rather circuitous evidence, there is no information on the mechanism of action of UV radiation or the specificity of this effect. UV radiation may act by exposing antigens in damaged skin or inducing the formation of new, cross-reacting antigens. Isolation of the antigen responsible for the immune response in pemphigus might permit investigators to explore these two alternatives in a suitable animal model.

13.3.2. Bullous Pemphigoid

A subepidermal blister is the main feature of this disorder, and the line of separation has been localized to the space between the basal cells of the epidermis and the basal lamina. Circulating antibodies to antigens of the basement membrane zone are usually present, and a linear deposit of IgG and complement is present in that region of the skin. The disorder has not been reproduced in primates or *in vitro*.

Induction or exacerbation of bullous pemphigoid by exposure to sunlight or to artificial sources of radiation, such as those used in phototherapy, is well documented. The microscopic changes in the disease were reproduced in two patients exposed to UV radiation,¹⁹ and this was associated with a deposition of IgG and complement. It is interesting that neither alteration was detected 5 hr

after the exposure and that both were present 24 hr postexposure; thus, the question as to whether the deposition of immunoreactants precedes histologic change thus remains unanswered by that study.

Induction of bullous pemphigoid has been reported following topical application of 5-fluorouracil, an antimitotic and irritant that was being used for the treatment of actinic keratoses.²⁹ Blisters first appeared at the site of application, but soon became generalized. Thus, it is possible that any cause of a marked inflammatory response to the skin may trigger bullous pemphigoid in a predisposed individual. However, further studies of the effect of UV radiation in this disease would be useful not only to determine its mode of action, but also to study the time course of the development of changes in the skin.

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Chapter 14

Other Photodermatoses

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14.1. Introduction

There are two photodermatoses, polymorphic light eruption (PMLE) and actinic reticuloid-photosensitive eczema, that must be included in a discussion of clinical photoimmunology because the suggestion is often made that their pathogeneses involve disturbances of immune function. The etiology of these disorders is unknown and, in addition, little is known about their pathogeneses. However, considerable work has been directed toward investigating the supposed immune nature of these diseases, and the results of these investigations will be reviewed because they may provide a basis for further work.

14.2. Polymorphic Light Eruption

Polymorphic light eruption is a delayed abnormal cutaneous response to electromagnetic radiation that appears from 2 hr to 5 or more days after exposure and lasts days or weeks. The rash varies in appearance among patients and may consist of small papules, plaques, or eczema. Unfortunately, the condition has not always been defined clearly, and it is quite possible that more than one disease is included under this label. For example, in some series of patients reported to have PMLE, there has been a high incidence of atopy. This may indicate the inclusion of patients with photosensitivity and atopy, a condition probably unrelated to PMLE. Similarly, eczematous PMLE appears to be very common in some geographic locations, but many of these patients may have a photoallergic reaction to an unidentified environmental allergen. The papular variety of PMLE is most common. This typically commences in young adult life and exhibits some predilection for females. The condition probably lasts for life, but since most cases are mild, slight alteration of lifestyle with limitation of exposure to sunlight enables patients to avoid triggering the eruption. A family history of PMLE is ob-

tained in some patients, but there is no definite evidence that this occurs with greater frequency than would be expected with a common condition.

The fundamental cause of PMLE is unknown; however, exposure to radiation clearly is involved in the pathogenesis. The most favored theory is that the pathogenesis includes an interaction between such radiation and an "allergic" mechanism. Several features of the condition are often cited to support an immune pathogenesis including (1) a delayed reaction time for the appearance of the eruption, (2) a perivascular lymphocytic infiltrate in the dermis seen on histologic examination of lesions, (3) appearance of the eruption at nonexposed but previously involved sites following sun exposure, and (4) in some patients, the association of solar urticaria with PMLE (see Chapter 11). These features separately or collectively are not very strong evidence to support an immune basis for the disease, but almost all studies of the pathogenesis of PMLE have been directed towards exploring this possibility.

Positive passive transfer of a PMLE-like eruption was reported by Epstein in 1942.¹ Serum from a patient with eczematous PMLE was injected into a normal subject and the site of injection was exposed to sunlight, resulting in a papular eruption at the site 9 days later. This was interpreted as indicating that an antigen-antibody reaction was responsible for the disease. Such a reaction would, of course, be inconsistent with the delayed character of the response. In a subsequent study, attempts to passively transfer the reaction by using sera obtained from 13 patients with PMLE gave uniformly negative results.² Another unsuccessful attempt to transfer the reaction involved a local injection of peripheral blood leukocytes obtained from one patient with PMLE and subsequent exposure of the injection site to radiation³; details of the technique were not included in the report.

The possibility of an autoimmune process underlying the disease has also been explored, with most attention being directed at a possible relationship between PMLE and lupus erythematosus (LE). At first glance, such an association is attractive because photosensitivity is common in LE and it is sometimes very difficult to distinguish between the rashes in PMLE and LE on the basis of morphologic features. A serum antinuclear antibody (ANA) was found in one-third of patients with PMLE that were tested using an immunofluorescent tumor imprint technique specific for the detection of the antibody.⁴ However, when a more conventional substrate was used in this test, results for circulating ANAs were negative in all patients.⁵ The LE cell test, which is more specific for lupus than the ANA test, was also found to be negative in a group of patients tested.⁶ Furthermore, examination of biopsies of skin from patients with PMLE by means of direct immunofluorescence did not reveal deposits of immunoglobulin; these deposits are a diagnostic feature of LE.^{5,7} Therefore, apart from the occasionally similar morphology of the eruptions in PMLE and LE, there is nothing to suggest that the two diseases are related. There has also been interest in levels of circulating immunoglobulins because of reports that serum IgE and IgM levels

are elevated in some patients with PMLE,⁶ but this has not been a constant finding.⁸

Lymphocyte function in patients with PMLE has been another area of research interest. Following exposure to UV radiation in vitro, lymphocytes from patients with PMLE differed from lymphocytes from normal control subjects by having a higher level of S-phase DNA synthesis and RNA synthesis, a lack of unscheduled DNA synthesis, and a greater response to stimulation by phytohemagglutinin (PHA).^{9,10} Both of these studies, which are from the same center, are very difficult to interpret because descriptions of methodology lack detail. Furthermore, similar studies by other groups have failed to support the findings.^{8,11} In an in vitro assay, leukocytes from patients with PMLE were unresponsive to stimulation by homogenates of the patient's irradiated and nonirradiated skin.¹²

What do all these observations mean? Perhaps the most striking feature of these studies is that for each positive result reported, at least one study has failed to support the findings. Therefore, although some clinical and histologic features of PMLE suggest an immune pathogenesis, there is, to date, no scientific evidence to support this hypothesis. To understand the pathogenesis of PMLE, it is first necessary to define the disease and determine whether it represents one or several conditions. Second, perhaps it is time to consider a nonimmunologic pathogenesis. The only study that has reported positive results in this direction found abnormal urinary excretion patterns of tryptophan metabolites in some patients with PMLE,¹³ suggesting that PMLE is caused by a defect of tryptophan metabolism. This idea does not appear to have been explored further.

14.3. Actinic Reticuloid-Photosensitive Eczema

Actinic reticuloid is a term introduced by Ive et al.¹⁴ to describe a chronic dermatosis characterized by thick plaques on exposed areas that is associated with marked photosensitivity to UVB, UVA, and, at times, visible radiation. A dense lymphocytic infiltrate, including some atypical cells, is present in the dermis and invades the epidermis. Photosensitive eczema¹⁵ is characterized by a chronic eczema of the exposed skin and a marked photosensitivity restricted to the UVB spectrum. Both conditions only occur in middle-aged or elderly males, and often there is a past history of some other nonphotosensitive skin disorder. Recently, the suggestion has made that these two conditions are one disease because patients have been observed to have the features of both diseases at different times and because some patients may have the clinical features of one disorder with the photobiological characteristics of the other disorder.^{15,16} The term *photosensitivity dermatitis-actinic reticuloid (PD/AR) syndrome* has been used to describe the disease¹⁷; it is hoped that a somewhat more abbreviated name might eventually be introduced.

The etiology of these diseases is largely unknown, but some exogenous factor is suggested by the marked geographic clustering of cases in the United Kingdom; in contrast, only two or three cases have been reported from North America. Photopatch testing with known photosensitizers has yielded negative results in most studies. However, PD/AR syndrome can bear a close clinical resemblance to the persistent light reactor state, which suggests that an unsuspected photoallergen might be involved. The possibility that plants may be involved in its pathogenesis has recently been raised. Most patients exhibit contact hypersensitivity to various oleoresin extracts from compositae plants, as revealed by patch tests.^{18,19} Contact and possibly photocontact hypersensitivity to extracts of lichens has also been reported in some patients.²⁰ These plant extracts are phototoxic in the *in vitro* photohemolysis assay,^{19,21} but these results do not show a close correlation with photosensitivity *in vivo*. It is possible that these compounds are not the primary sensitizers in patients with PD/AR syndrome, but instead that they may be cross-reacting with an unidentified sensitizer that produces both contact and photocontact allergy. Evidence has also been presented that kynurenic acid, a metabolite of tryptophan, may be involved in the pathogenesis of this syndrome. Particles that have spectrophotometric properties similar to those of kynurenic acid were found in the cells of sweat glands in a patient with this condition,²² and an abnormal excretion pattern of kynurenic acid and other tryptophan metabolites has been observed after oral tryptophan challenge in these patients.²³

There are two reports of patients with actinic reticuloid developing a lymphoma.^{24,25} This may have been only a chance association, or, alternatively, these patients may have had lymphoma associated with photosensitivity, which is a clinical association that occurs occasionally. The latter possibility is unlikely because of the time interval of several years between the development of photosensitivity and the appearance of the lymphoma.

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Chapter 15

Phototherapy and Photochemotherapy

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15.1. Introduction

The beneficial and adverse effects of nonionizing radiation in the treatment of certain skin diseases may involve immunologic mechanisms. The most recent and direct evidence for this comes from studies of the effects of oral psoralen photochemotherapy (psoralen plus UVA radiation, PVA), which show that immune function is affected by this treatment. Furthermore, the immune system is involved in the pathophysiology of most diseases found to respond to PUVA therapy. Thus, suppression of various skin diseases by this treatment may be produced by alteration or suppression of the immune responses that mediate them. Some of the adverse effects of this treatment (in particular, skin cancer) also could be due in part to alterations of immune function. UVB phototherapy has been used in the treatment of skin disease for many years, but there has been little interest in its potential immunologic effects. However, further study is warranted because various specific alterations of immune responses have been observed in animals exposed to UVB radiation.

15.2. Treatment Regimens

Several treatment systems involving UV radiation have been used in the therapy of skin disease. The most commonly used treatments are presented here.

15.2.1. Goeckerman Regimen

Introduced by Goeckerman in 1925,¹ this treatment involves exposure to UV radiation and application of crude coal tar to the skin. It is most often used to treat psoriasis, but it is also used to treat eczema and mycosis fungoides. Patients are usually hospitalized for 3 to 4 wk and treated daily with two appli-

cations of tar and one exposure to UV radiation. Psoriasis may recur within a few months of treatment, and in some patients, as many as 3 or 4 treatment programs may be required each year to maintain reasonable control of the disease.

Crude coal tar is a photosensitizer and antimitotic agent. Until recently, the mechanism of action for the Goeckerman regimen was usually assumed to involve tar photosensitization. However, the photobiologically effective component of the emission spectrum of radiation sources used in the treatment usually is in the UVB waveband, whereas tar photosensitization is induced by exposure to UVA radiation. Furthermore, recent studies have examined the role of tar and found that tar photosensitization is not an essential component of the treatment as usually administered. If repeated erythemogenic doses of UVB radiation are used, the addition of tar produces no further detectable therapeutic benefit.^{2,3}

15.2.2. UVB Phototherapy

UVB phototherapy, which is primarily used to treat psoriasis, involves repeated exposures to fluorescent bulbs or medium-pressure mercury arc sources of radiation. Although the emission spectra of the various radiation sources used include the UVC and UVA wavebands, the most biologically effective wavelengths are in the UVB region. Since it was shown that tar is not an essential component of the treatment of psoriasis, there has been renewed interest in UVB phototherapy, particularly as a home or ambulatory treatment for skin disease. Its convenience may lead to the widespread use of maintenance therapy aimed at preventing recurrence of the disease; however, regular exposures to UVB radiation required for maintenance therapy may result in individual patients receiving large cumulative doses of UVB radiation.

UVB phototherapy usually involves 3 to 5 exposures each week during the clearance phase of treatment and then 1 to 2 exposures per week for maintenance therapy. A treatment usually consists of whole-body exposure in a cabinet lined with fluorescent sunlamps.

15.2.3. PUVA Therapy

Introduced for the treatment of psoriasis by Parrish et al. in 1974,⁴ PUVA therapy involves the ingestion of a photosensitizer, 8-methoxysoralen (methoxsalen), followed 2 hr later by exposure to UVA radiation. Usually the whole body is exposed to radiation in a square or cylindrical radiator lined with UVA bulbs. To achieve clearance of psoriasis, most patients require about 20 treatments over 4 to 10 wk. Maintenance treatment is then commenced at a frequency of 1 to 4 treatments each month. In some centers, 8-methoxysoralen is administered topically rather than orally, usually by having the patient bathe in a very dilute solution of the photosensitizer a few minutes before exposure to radiation.

15.3. Clinical Applications

UV radiation produces a beneficial effect in a variety of diseases (Table 15.1), but is most commonly used in the treatment of psoriasis. This skin disease is familial and affects 2 to 5% of the population. The pathophysiology of psoriasis is not entirely clear, but the most obvious changes in the skin are vascular dilatation and rapid proliferation of basal cells in the epidermis, resulting in marked epidermal hyperplasia and thickening of the stratum corneum. Morphologically, these abnormalities are expressed as erythematous plaques covered with a fine silvery scale. The pathogenesis of psoriasis is unknown, but recent interest in this subject has focused on the immunologic abnormalities associated with the disease. A wide variety of such changes have been reported, including deposits of immunoglobulin and complement in cutaneous vessels and the epidermis, presence of antibodies that react with antigens in the stratum corneum, defective function and decreased numbers of circulating T lymphocytes, deficient suppressor T lymphocyte activity, abnormal monocyte function, and impaired delayed hypersensitivity responses in the skin. These preliminary observations require confirmation and further study, but they do raise the possibility that psoriasis could have an immunologic pathogenesis and may belong to the group of autoimmune diseases.

Atopic eczema is a disorder that responds to PUVA therapy, but not to UVB phototherapy.⁵ The response of atopic eczema to PUVA therapy differs

Table 15.1
Effectiveness of Phototherapy in Skin Diseases

Disease	PUVA therapy	UVB phototherapy
Psoriasis	+	+
Mycosis fungoides	+	± ^a
Lichen planus	+	- ^a
Vitiligo	+	-
Alopecia areata	±	-
Atopic eczema	+	-
Seborrheic eczema	±	-
Solar urticaria	+	±
Polymorphic light eruption	+	+
Photosensitive eczema	+	-
Actinic reticuloid	+	-
Urticaria pigmentosa	+	-
Pityriasis lichenoides chronica	+	-

^a(+) Effective in controlled studies; (±) effective in uncontrolled studies or anecdotal reports; (-) ineffective or untried.

from that of psoriasis in that much more treatment is required to achieve clearance of the disease and also to maintain control of the disease. This familial disorder is associated with hay fever and asthma and is thought to have an immune pathogenesis. Most patients have evidence of increased activity of the humoral immune system; most notably, raised serum levels of IgE. Various defects of T lymphocyte function have also been described, including decreased numbers of circulating suppressor T lymphocytes, which may explain the increased activity of B lymphocytes.

Vitiligo, another disorder that responds to PUVA therapy but not to UVB phototherapy, is characterized by a selective loss of melanocytes from the epidermis that results in patches of depigmentation. The skin is normal except for the absence of melanocytes. Most evidence as to the pathogenesis of vitiligo points toward it being, at least in part, an autoimmune disorder: It is frequently associated with other autoimmune diseases, it can be accompanied by halo nevi, antibodies to melanocytes are detectable in some patients, and a lymphocytic infiltrate is present in the dermis and epidermis in early cases.

A number of photodermatoses have been found to respond to PUVA therapy, including solar urticaria, actinic reticuloid, photosensitive eczema, and polymorphic light eruption (PMLE) (see Chapters 11 and 14). The pathogeneses of these diseases are largely unknown, but some evidence points toward involvement of the immune system. The mechanism of action of PUVA in photodermatoses is unknown, but may involve protection by melanogenesis and thickening of the stratum corneum, depletion of some mediator that is involved in these disorders, or alteration of some other pathologic response which triggers the diseases.

Lichen planus and mycosis fungoïdes are characterized by infiltration of the skin by normal or neoplastic lymphoid cells; both respond to PUVA therapy. In lichen planus, T lymphocytes are found in a bandlike infiltrate in the dermis immediately beneath the epidermis, and the histologic appearance has been likened to a delayed hypersensitivity response. However, apart from the morphologic similarity to other immunologic disorders, there is no evidence that a specific immune response is involved in this disease. Psoralen plus UVA radiation therapy clears the rash and eliminates the lymphocytic infiltrate from the skin. Mycosis fungoïdes is a T cell lymphoma that can remain localized to the skin for many years, but eventually it spreads to involve systemic organs. The neoplastic lymphoid cells form an infiltrate in the dermis and also invade the epidermis. Psoralen plus UVA radiation therapy clears the rash of mycosis fungoïdes and also eliminates the neoplastic cells from the skin, but continued treatment is necessary for suppression of the disease. There is no evidence that PUVA therapy alters the course of the disease.

The spectrum of diseases that have been found to respond to PUVA therapy is obviously quite broad. In contrast, of the diseases listed, only psoriasis and PMLE have been shown to respond to UVB phototherapy. However, the thera-

peutic activity of UVB phototherapy has not been carefully evaluated, and quite possibly it may be effective in other disorders. In particular, assessing the effect of UVB phototherapy in lichen planus and mycosis fungoides would provide further information on the toxicity of this waveband for lymphoid cells.

15.4. Immunologic Effects

15.4.1. Lymphocytes

Exposure of human peripheral blood lymphocytes *in vitro* to 8-methoxysorafen plus UVA radiation or UVB radiation results in a dose-dependent decrease in the viability and function of these cells. Because lymphocytes percolating through the blood vessels and interstitial spaces of the dermis are within the depth of skin penetrated by both these types of radiation, it might be expected that UVB phototherapy and PUVA therapy could directly alter the function and possibly the viability of lymphocytes *in vivo*. Indeed, since the average circulation time for blood is about 10 min and treatments may last up to 45 min, a single treatment could be expected to result in the exposure of most circulating lymphocytes. Of course, apart from any direct toxic effect of the radiation on lymphocytes, mediators of inflammation and photoproducts produced in the skin may have an indirect effect on the viability and function of lymphocytes. Studies of single exposures cannot provide complete information on the immunologic effects of radiation on lymphocytes. Some patients receive hundreds of exposures (particularly with PUVA therapy) in order to maintain control of their disease, and a single exposure may produce only a transient alteration of lymphocyte function, whereas the cumulative effect of repeated insults to lymphocytes and other components of the immune system may produce long-term alterations.

Most investigators who have examined the effect of PUVA therapy on lymphocytes *in vivo* have found changes, but results are varied. Immediately following exposure to UVA radiation during a course of PUVA therapy, DNA synthesis was diminished in unstimulated peripheral blood lymphocytes obtained from patients with psoriasis.⁶ Levels of circulating T lymphocytes, as identified by the formation of E rosettes, were decreased after several exposures to PUVA therapy in patients with psoriasis,^{7,8} but they returned to normal as treatments continued. The percentage of cells that formed E rosettes was also decreased in the peripheral blood of patients with various other diseases who were receiving PUVA therapy.⁹ However, another study failed to find any alteration of circulating T cells during short courses of PUVA therapy.¹⁰ In further contrast, the percentage of E-rosette-forming cells in the peripheral blood of patients with psoriasis was increased during a course of PUVA therapy.¹¹ In patients with

psoriasis, the proportion of circulating B lymphocytes, identified by the presence of surface membrane immunoglobulins, was unchanged by PUVA therapy in several studies.^{7,9,10} The response of lymphocytes to phytohemagglutinin (PHA) has been found to be unaffected^{11,12} or diminished^{10,13} early in the course of therapy. These apparent conflicts between the results of different studies are probably caused by several factors. Psoriasis alone affects the number of circulating E-rosette-forming cells, the number being diminished in patients with active disease.^{8,11} The time of blood collection relative to the last exposure to PUVA varied between studies, and in some studies was not specified. Finally, PUVA therapy is not standardized and presumably the doses administered to patients varied a great deal. In normal subjects, we have found that a single whole-body exposure to PUVA produces dose-dependent changes in lymphocyte function that last for hours.

Over the past few years, we have been monitoring the effects of PUVA therapy on lymphocyte function in patients with psoriasis to determine whether alterations appeared with progressively higher cumulative exposure to this treatment. During the first 2 wk of PUVA therapy, lymphocytes obtained 1 to 3 days after the last exposure to PUVA showed an impaired response following stimulation with PHA. However, this impairment did not persist and was not seen during subsequent weeks of treatment. Also, lymphocytes obtained 1 to 7 days after PUVA exposure from patients who had been treated for up to 2 yr showed normal function. Recently, we studied ten patients who had received more than 200 treatments over a 2 to 6 yr period, with cumulative exposure doses of 1700 to 6000 J/cm² UVA radiation.^{14,15} These patients were selected on the basis of their having marked premature aging of the skin. Blood samples were taken 4 to 7 days after the last PUVA treatment and a variety of tests of lymphocyte function were performed. The results were compared to those obtained with lymphocytes from untreated patients and normal control subjects. At suboptimal doses of PHA, concanavalin-A (con-A), and pokeweed mitogen (PWM), there were abnormally low responses in all ten patients tested with at least one mitogen and in seven patients tested with two mitogens. At optimal stimulating doses of mitogens, there were abnormally low responses to at least one mitogen in seven patients. Three patients had abnormally low responses following stimulation with both optimal and suboptimal stimulating doses of all three mitogens. Blood lymphocytes from these ten patients were also analyzed by flow cytometry with monoclonal antibodies to T cell surface markers OKT3 (all T cells), OKT4 (helper/inducer cells), OKT6 (common thymocytes), and OKT8 (suppressor/cytotoxic cells). The percentages of T3- and T4-bearing cells were reduced in the PUVA-treated patients compared to normal controls and patients with active psoriasis. A correlation was observed between the results of mitogen and T cell subset studies in that a low level of T3- and T4-bearing cells was found in patients who had low lymphocyte responses to PHA and PWM. These findings suggest that long-term PUVA therapy has an immunosuppressive effect manifested by a re-

duced response of lymphocytes to mitogen stimulation and a selective loss of the helper/inducer T cell population. A prospective study is required to monitor lymphocyte function before, during, and after several years of PUVA therapy to confirm these observations. Also, further studies are required to determine the duration of this defect after treatment is stopped; however, these studies would be difficult because patients are unwilling to stop all treatment for their psoriasis and the alternatives to PUVA therapy, such as methotrexate, may have effects on lymphocytes that would make the interpretation of the study difficult.

The overall picture of the effect of PUVA therapy on lymphocytes is becoming clearer. After a single or a few exposures, PUVA therapy produces effects that appear to be transient and dose related. These changes have been most clearly documented in normal subjects (see Chapter 8). After years of exposure, PUVA therapy also produces cumulative changes that may or may not be transient, and these effects are probably also related to total dose of therapy.

The effect of UVB phototherapy on lymphocytes has attracted little interest. However, this is an important question because concern about possible adverse immunologic effects of PUVA therapy is already leading to the use of UVB phototherapy as a "safer" alternative treatment. Furthermore, as mentioned previously, the approach to UVB phototherapy of psoriasis is undergoing some dramatic alterations with regard to frequency of use. High exposure doses over years of UVB phototherapy combined with environmental exposure to sunlight might have a deleterious effect on lymphocytes. In a preliminary study, lymphocyte function was tested in ten patients with psoriasis who had received high-dose UVB phototherapy in the clearance phase of treatment, followed by maintenance treatment for 6 to 18 months. Lymphocyte function in the UVB-treated patients was similar to that in normal control subjects and untreated patients with psoriasis. There were no differences in the lymphocyte responses to graded doses of PHA, con-A, or PWM, and the distribution and number of circulating subpopulations of B and T lymphocytes were similar in the three groups (unpublished observations). However, this study must be extended because the period of observation is short, and it is quite possible that treatment with UVB phototherapy over several years will result in changes of lymphocyte function as has been observed in PUVA-treated patients.

15.4.2. Delayed Hypersensitivity Responses

Preliminary findings suggest that PUVA therapy may result in impairment of delayed hypersensitivity responses in the skin. Following sensitization with dinitrochlorobenzene (DNCB), a contact allergen, the responses to an elicitation dose of the allergen were diminished in PUVA-treated patients in comparison to the responses seen in untreated psoriatic patients and normal control individuals.¹⁶ A semiquantitative assessment of the elicitation responses was made in that study, and a correlation between the skin type of the patients and the extent of

depression of the response was found: The greater the tanning response of the individual, the more normal was the response. The assumption was made that the skin type of the individual would influence the dose of radiation "received by the skin," but this assumption may not be valid because patients with good tanning responses are given higher exposure doses of UVA radiation to compensate for melanin absorption. The main problem with the study was the lack of adequate controls; only nine untreated patients with psoriasis were studied and compared with 102 patients treated with PUVA. Another report¹⁷ supported some of the findings of that study in that impaired responses to DNCB were found in psoriatic patients treated with PUVA therapy and a similar correlation with skin type was seen. However, in comparison with healthy control subjects, untreated patients with psoriasis also showed a diminished response to DNCB, which suggests that the disease itself may play a role in the impairment of delayed hypersensitivity. In both these studies,^{16,17} both sensitization and elicitation of contact hypersensitivity were apparently performed on skin that had been exposed to UVA radiation; therefore, it is not possible to distinguish between a local and systemic effect of PUVA therapy. A prospective study is required that uses patients as their own controls by testing them before the start of PUVA therapy and then retesting them during treatment with the same allergen and another noncross-reacting allergen.

Contact sensitivity to nitrogen mustard was found to be diminished following a course of PUVA therapy in five patients with mycosis fungoïdes who had previously shown marked sensitivity to this chemical.¹⁸ This study was uncontrolled, and the disease state, which alone is known to impair immune responses, may have been responsible for the diminished cutaneous hypersensitivity. Patients with psoriasis and mycosis fungoïdes have also been deliberately exposed to UVB phototherapy and PUVA therapy in an attempt to prevent or delay the development of sensitivity to nitrogen mustard.¹⁹ The time required to develop sensitivity was delayed in the group exposed to UV radiation in comparison to patients not exposed to radiation, and possibly the number of patients sensitized during the period of treatment was also lower. However, there was no set protocol for the UV radiation treatment, the numbers of patients were small, and the area of application of nitrogen mustard varied considerably, so that it is very difficult to interpret the results. Similar criticisms can be made of another study in which contact-allergic and contact-irritant reactions were found to decrease following a course of PUVA therapy in three patients.²⁰

Psoralen plus UVA radiation therapy has been found to alter the membrane properties of Langerhans cells.²¹ Repeated treatments resulted in a reduction of the number of Langerhans cells that could be identified by means of the ATPase stain, and this abnormality persisted while treatment continued. Unfortunately, in this study, no attempt was made to correlate the defect of Langerhans cells with contact hypersensitivity responses.

All of these observations can only be regarded as suggestive that PUVA therapy, and possibly also UVB phototherapy, can inhibit the development and/or expression of contact hypersensitivity responses in the skin of humans. Adequately controlled studies are required to confirm the effect of the treatments, to examine whether the effects are local (at the site of exposure) or both local and systemic, and to determine whether the depression of hypersensitivity is mediated by way of an effect on induction or elicitation.

15.4.3. Other Immunologic Functions

Most interest has focused on the effects of PUVA therapy on lymphocytes and contact hypersensitivity, and there are few studies of other aspects of immune function. The locomotion of polymorphonuclear leukocytes from normal subjects was found to be inhibited by treatment of the cells with PUVA in vitro.²² However, the locomotion of both polymorphonuclear leukocytes and mononuclear cells from patients with psoriasis before and after a course of PUVA therapy was similar.²³ Enhanced locomotion of both cell types was found in patients with psoriasis when compared to healthy controls.

15.5. Clinical Effects of Immune Alterations

Since PUVA therapy appears to be immunosuppressive for lymphocyte function and delayed hypersensitivity responses, consideration must be given to whether this alteration results in any adverse effects. Nonspecific immunosuppression might be manifest as an increase in the incidence of infections and neoplasia. Herpes simplex infections of the face and other exposed areas are certainly a common occurrence in patients receiving PUVA therapy, but they also are common following sun exposure and during UVB phototherapy. The mechanism by which this infection develops is unknown, so it is not possible to relate this increased incidence to a depression of immune function. Other infections do not appear to be more common in patients receiving PUVA therapy. However, this comment is only anecdotal because no prospective studies have been conducted; there could be a marked increase in the frequency of common viral infections without the problem becoming obvious clinically. There are also no reports of an increased incidence of systemic neoplasia in patients treated with PUVA. This has been examined in 1380 patients closely followed for 5 yr in a multicenter study of PUVA therapy (NIH PUVA Follow-up Study²⁴; however, this period of observation may be too short to fully assess this risk. The incidence of cutaneous neoplasia was found to be increased in that study.

Specific alterations of immune function induced by PUVA therapy may also give rise to adverse effects. For example, PUVA therapy could produce changes

analogous to the effects of UVB radiation in mice, in which specific alterations of immune responsiveness favor the development of skin cancer (see Chapter 8). Production of new antigens as a result of photon damage is another possible specific change that could lead to the development of autoimmune disease such as lupus erythematosus and pemphigoid.

15.5.1. Skin Cancer

Psoralen plus UVA radiation treatment is mutagenic in a variety of systems, including those using mammalian cell lines. The treatment is also carcinogenic in mice and causes the development of both squamous cell carcinoma (SCC) and fibrosarcoma in the skin. Therefore, it is not surprising that PUVA treatment appears to be associated with skin cancer in man. This adverse effect of the treatment appeared after 2 yr of follow-up of patients in the NIH PUVA Follow-up Study,²⁵ and after a 5-yr period of observation, the incidence of skin cancer has persisted. The increased incidence applies only to SCC; the incidence of basal cell carcinoma (BCC) and melanoma is not significantly higher than in the general population. Thus, the ratio of BCC to SCC in the general population is 3:1 or greater, but in patients treated with PUVA, the ratio is reversed. Squamous cell carcinomas are approximately 10 times more frequent in patients treated with PUVA than in normal geographically matched populations. The cancers are morphologically similar to sun-induced neoplasms, but they occur mainly on the trunk and lower extremities, areas that are infrequently involved with skin cancer from sun exposure. Furthermore, in some patients, multiple lesions develop over a period of a few months; this is very unusual with sun-induced lesions. The increased incidence of SCC in patients treated with PUVA is mainly due to a very high incidence of SCC in patients who were exposed previously to ionizing radiation for skin disease or who received frequent treatments with tar and UVB radiation. In these groups of patients, a dose-response relationship has been observed: The frequency of skin cancer increases with exposure to PUVA therapy. The short latency period for development of PUVA-associated neoplasia and the interaction of PUVA with other carcinogens suggests that PUVA may not be a primary carcinogen in these patients, but instead may be acting as a promoter for a carcinogen.²⁶

An increased incidence of skin cancer was also observed in another prospective study of more than 400 PUVA-treated patients followed for a period of up to 5 yr²⁷; however, this increase in incidence was small and was confined to patients previously exposed to arsenic or ionizing radiation. This study included a much higher proportion of patients of dark skin type than that included in the NIH Follow-up Study, and since the risk of PUVA-associated skin cancer is greatest in fair-skinned patients, this difference probably explains the discrepancy between the two studies.

There have been isolated case reports of Bowen's disease, BCC, and keratoacanthomas occurring in patients treated with PUVA, but apart from some unusual features in terms of site of occurrence and development of multiple lesions, the evidence linking these neoplasms to the treatment is inconclusive.

The reports of skin cancer being a risk factor in PUVA therapy have not been universally accepted. A prospective study²⁸ of more than 500 patients followed for up to 4 yr did not find an increased frequency of skin cancer. It has also been claimed that the increased frequency of SCC in patients treated with PUVA is caused by other carcinogens alone and that patients with psoriasis are particularly prone to develop skin cancer,²⁹ but there is no evidence to support the latter idea. In fact, until recently it was a widespread belief that psoriatic patients were somehow more resistant to skin cancer than nonpsoriatic individuals.

The main defect in the NIH Follow-up Study was its lack of a control group of matched patients with psoriasis who have not been treated with PUVA therapy. Since PUVA therapy is now widely available, it would have been difficult, if not impossible, to maintain such a control group. Although this defect has led to reasonable criticism, the reversal of the SCC to BCC ratio, the preponderance of lesions on areas of the body that are not the usual site of sun-induced lesions, the temporal relationship of development of lesions to initiation of therapy, and the dose-response effect now being observed all argue strongly for an association of PUVA therapy with skin cancer.

There have been no detailed studies of the immune status of PUVA-treated patients who have developed skin cancer. In the studies of lymphocyte function following long-term PUVA therapy,^{14,15} two patients who showed a deficiency of helper/inducer lymphocytes in the peripheral blood and a low response of their lymphocytes to mitogens developed multiple SSCs soon after these defects were found. However, this may have been a chance association, or the developing lesions may have been responsible for the defects of lymphocyte function. Prospective studies of patients using both in vitro assays and in vivo tests of immune function are required to determine whether immune defects are associated with the development of skin cancer.

Sunlight is well-established as an etiologic agent in the induction of skin cancer, and studies of the action spectrum for production of skin cancer in animals indicate that the UVB waveband is responsible for these lesions. Despite this evidence, there has been little interest in assessing the risk of producing skin cancer in psoriatic patients as a result of UVB phototherapy. A potential problem in such studies is that most patients have also been treated with tar, a well-known carcinogen, in addition to UVB radiation, so that it is difficult to determine the relative importance of the two agents. In one study,³⁰ exposure of patients with psoriasis to tar and UV radiation was found to be associated with an increased incidence of skin cancer, but in another study,³¹ the incidence was similar to that in the general population. The introduction of high-dose UVB phototherapy

without tar will provide an opportunity for a clear evaluation of the effect of UVB radiation alone on the incidence of skin cancer.

15.5.2. Systemic Lupus Erythematosus

UVB radiation can induce skin lesions in patients with lupus erythematosus (LE), and sun-induced induction and exacerbation of both cutaneous and systemic LE is common. The mechanism of these changes is not clear, but one possibility is that UV-radiation-induced alterations of the antigenicity of DNA have a pathogenic role in such cases. There are no published reports of UVB phototherapy being responsible for the induction or exacerbation of systemic LE during therapy of skin disease, but it is likely that this has occurred. Furthermore, there have been no studies of the frequency of development of a positive antinuclear antibody (ANA) test during the course of UVB phototherapy.

Psoralen plus UVA radiation alters DNA structure, and it has been shown that the photoproducts resulting from this interaction are antigenic in rabbits.³² Therefore, a theoretical concern is that PUVA therapy could either predispose otherwise normal individuals to the development of systemic LE or trigger the condition in a susceptible individual. There have been three case reports³³⁻³⁵ of LE or a similar syndrome developing in patients that have been treated with PUVA therapy. This frequency does not suggest anything more than chance association of two reasonably common events. In a 2-yr study of over 1000 patients receiving PUVA therapy,³⁶ there was no increase in the frequency of a positive ANA test when first and last tests were compared. Furthermore, the finding of a positive result on repeated testing was found to be related to the number of tests performed and not to the amount of exposure to PUVA. However, in a study of 99 patients,³⁷ the frequency of positive ANA tests was increased and there was a significant correlation with the duration of PUVA therapy. Antibodies to DNA were not associated with the development of positive tests for ANA in that study.

The studies reported so far indicate that systemic LE is not a significant risk of PUVA therapy and that an association between PUVA therapy and the development of a positive ANA test is doubtful. Performance of a standard ANA test in patients treated with PUVA may be irrelevant because the correct substrate, namely PUVA-treated cells, is not used. A prospective study of patients to determine whether the development of an ANA specific for PUVA-treated tissue would be of interest for our understanding of LE.

15.5.3. Bullous Pemphigoid

Exposure to UVB radiation can induce lesions in patients who have bullous pemphigoid. In addition, induction of this disease has been observed as an adverse effect of UVB phototherapy for psoriasis.³⁸ There are also reports that

PUVA therapy can be associated with both reactivation and induction of bullous pemphigoid.^{39,40} Two patients with a history of the disease developed lesions while receiving PUVA therapy, and new blisters continued to appear after the treatment was stopped. A third patient developed the disease for the first time after initiation of PUVA therapy, and the disease promptly remitted once the treatment was discontinued. From these few observations, it appears that both UVB phototherapy and PUVA therapy can occasionally trigger bullous pemphigoid; these treatments are obviously contraindicated in patients with a history of the disease. Circulating antibodies to basement membrane antigen appear to be involved in the pathogenesis of this disease, and deposition of immunoglobulin and complement at the basement membrane is associated with the development of blisters. Psoralen plus UVA radiation and UVB radiation may be responsible for production of the basement membrane antigens. Alternatively, the radiation may be acting nonspecifically to expose existing antigens.

15.6. Mechanisms

The molecular and cellular mechanisms mediating the beneficial effects of photons are not known with certainty for any skin disease. UVB radiation suppresses scheduled DNA synthesis and is toxic to cells, particularly rapidly dividing cells. Therefore, in a disease such as psoriasis in which the epidermis is proliferating at a much faster rate than normal, UVB phototherapy may produce a beneficial effect by suppressing cell proliferation. Because PUVA treatment also is toxic to cells and suppresses DNA synthesis, it has been assumed it too controls psoriasis by inhibiting cell proliferation. A direct toxic effect of PUVA on the cell infiltrate could also explain its effectiveness in lichen planus and mycosis fungoides, disorders characterized by a lymphoid infiltrate in the skin. Because PUVA therapy is also beneficial in a number of diseases that are not characterized by cell proliferation or infiltration (such as atopic eczema, vitiligo, and solar urticaria), other mechanisms of action of this treatment also must be considered.

An understanding of the pathogeneses of the diseases being treated is very helpful for characterizing the mechanism of action of the treatment. Unfortunately, we only have a sketchy knowledge of the pathogenesis of most of the skin diseases that have been discussed. There is some indication that solar urticaria and vitiligo have an immune pathogenesis and that atopic eczema is immunologically mediated. Psoralen plus UVA radiation treatment can produce some alterations of immune function; therefore, it is quite possible that this treatment is effective in these diseases because it alters the immune reactions involved in their pathogeneses. It has been observed that, in each of these three diseases, areas of skin shielded from UVA radiation during PUVA therapy continue to express the disease after exposed areas have become free of disease. Thus, any effect of PUVA therapy must be exerted locally in the skin at the site of treatment.

Because so little is known about the nature of the immune reactions occurring in the skin in these three diseases, it is only possible to speculate on what alterations PUVA therapy may produce. A T-cell defect appears to underlie atopic eczema; therefore, PUVA treatment may act by eliminating from the skin the lymphocyte subpopulation that is responsible for the disease or it may alter the reactivity of blood vessels or epidermal cells so that they no longer respond to the trigger for the disease. An alteration of mast cell function may be responsible for the effect of PUVA treatment in solar urticaria. In vitiligo, PUVA therapy may be toxic to the lymphocyte population that is thought to destroy melanocytes. Obviously, more information on the pathogeneses of these diseases is required before the mechanism by which PUVA therapy acts can be understood.

The beneficial effect of PUVA therapy in psoriasis could also be due to an alteration of immune responses, since evidence suggests an immune pathogenesis for this disease. Certainly it is very difficult to explain the response of psoriasis in the maintenance phase of PUVA therapy solely on the basis of suppression of DNA synthesis. As few as 1 or 2 treatments each month are usually all that is required to maintain a disease-free state, and yet suppression of DNA synthesis in the skin by PUVA lasts only 2 to 3 days at the most. Therefore, it is likely that PUVA therapy inhibits some other reaction that is responsible for initiating the increased cell proliferation; such a reaction could be immunologic in nature.

The mechanism of action of UVB phototherapy in psoriasis probably involves suppression of DNA synthesis in both the clearance and maintenance phases of treatment. Unlike the dosage of PUVA therapy, frequent exposures to UVB radiation are necessary to maintain a disease-free state. UVB phototherapy has not been adequately studied in other diseases to determine whether it is effective in disorders in which epidermal proliferation is not a feature, although it does not appear to be effective in atopic eczema or vitiligo.

15.7. Conclusions

A link between photon-induced alterations of immune function and the mechanism of action of phototherapy/photochemotherapy, in terms of both therapeutic effect and the production of adverse effects, is at present largely speculative. The two lines of evidence that suggest a link are: (1) Both therapeutic modalities can produce alterations of immune function in man and animals and (2) the pathogenesis of all diseases that respond to PUVA therapy are possibly immunologic in nature. Further study of the effects of the treatments, particularly in respect of immune responses on the skin, are required to elucidate their mechanism of action. Investigation of diseases in which there is a clearly defined pathogenesis involving an immune response, preferably with a suitable animal model, would be helpful in this regard.

Investigation of the role of photoimmunologic effects of PUVA therapy and UVB phototherapy are important because of the therapeutic opportunities that may result. If, indeed, these treatments act in part by an immunologic mechanism, it might be possible to enhance selectively the therapeutic effect and/or decrease the risk of adverse effects. For example, the action spectrum for the immunologic effect may be different from the action spectrum for carcinogenesis. In such a situation, use of a different waveband of radiation or of a photosensitizer other than psoralens may result in the same therapeutic benefit with less risk of adverse effects.

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