Schumacher

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Optical Properties of Human Skin

R. R. Anderson and J. A. Parrish

Despite the many years since optical spectra from human skin were first obtained, only recently have quantitative models of cutaneous optics been applied. This chapter aims to present the optics of human skin conceptually and quantitatively, to examine the structures and pigments that modify cutaneous optics, and to discuss current research in this area and its applications to photomedicine. Introductory sections on the structure of skin and on optical phenomena in turbid media are included in addition to the general introduction below. This chapter does not offer an exhaustive review of all studies related to the optics of human skin, but attempts to include those reliable studies pertinent to its goals. The interested reader can find thorough and more historical reviews in (1–3).

Unlike most other organs, the skin is accessible for visual inspection. Consequently, humans have learned to judge each other, in part, on the basis of pigmentation, texture, and cosmetic appearance of skin. The accessibility of skin often allows the dermatologist to diagnose and follow its condition simply by visual inspection. Specific local or generalized changes in skin color are most often due to abnormal structures or depositions of pigmented substances within the skin. As such, an understanding of the optical properties of the skin is helpful in explaining such changes and can be useful in diagnosis of skin or systemic disease. Quantitative optical measurements of skin in vivo can be used to quantify, monitor, and diagnose certain cutaneous and systemic conditions. For example, in phototherapy or photochemotherapy, in vivo spectral measurements of diffuse reflectance (remittance) can quantify

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the vascular and pigmentary responses of skin, and might also be used as an aid in determining optical dosimetry. Neonatal serum bilirubin levels can be monitored noninvasively by a similar technique. Patterns of visible and fluorescence of pigmented skin can be used to deduce the locality of abnormal quantities of melanin pigmentation.

In addition to making such diagnostic techniques feasible, a quantitative understanding of the optics of skin yields knowledge of the optical radiation doses received by different cell layers within the skin, by cutaneous blood, and by internal organs, when humans are exposed to optical radiation. Different wavelengths across the optical spectrum, defined here as approximately 250 nm in the ultraviolet to approximately 3000 nm in the infrared, reach vastly different depths within tissue. Because most photobiologic effects are both wavelength- and dose-dependent, the rational design and explanation of the effects of various phototherapies require knowledge of *internal optical dosimetry*. Our rapidly increasing knowledge of cellular and molecular photobiology gained from in vitro bacterial and tissue culture studies can in theory be related to observed responses of cells in situ by comparing the dose-related effects of optical radiation in vitro to those in vivo. Conversely, on the basis of knowing the practical upper limits of spectral radiant exposure doses experienced by cell layers, blood, or other structures in vivo, one can then concentrate on basic studies of repair, mutation, and metabolic changes induced by equivalent doses in vitro. The use of laser irradiation for selective thermal destruction of optically absorbing structures in skin, and control of the tissue depth affected by various forms of photochemotherapy, are examples in which understanding cutaneous optics allows selectivity and control of the damage induced.

Describing "the" optics of human skin is somewhat like describing "the" weather; it can be measured and understood, but cannot be considered static. The skin is a highly dynamic organ capable of withstanding, and mounting protective responses to, a host of deleterious environmental treatments and agents. As such, the optical properties of skin are also dynamic. Many different physical structures and chromophores (optically absorbing molecular structures) within different layers of the skin influence its optical properties over different spectral regions. Changes in vasodilatation, vascular permeability, oxygenation of cutaneous blood, bile pigments, carotenoids, melanin pigmentation, and thickness of any of the various layers of skin affect cutaneous optics. The simple act of bathing alters the optics of the stratum corneum to make one more sensitive to certain ultraviolet wavelengths. A 10-min exposure on a sunny day is sufficient in many persons to cause immediate pigment darkening—a photochemical oxidation reaction of melanin—subsequent delayed hyperpigmentation (tanning), and epidermal hyperplasia. Systemic and cutaneous pathologic conditions can markedly alter cutaneous optics as well.

The microscopically complex structure of the skin makes an ent rigorous analysis of its optics virtually impossible. The situation is however, too complicated to allow useful quantitative study of the optic skin. On the macroscopic scale, phenomenologic theories of radiation tra in turbid media can be applied to model the optics for each skin layer. M optical variables within each layer in vitro have been studied, allowing on conceptually consider the skin as a definable multilayered optical syst Optical interactions between each definable layer can then be considered produce a general and quantitative description of cutaneous optics. I tunately, many of the major chromophores are normally confined to a sir layer. Melanin, for example, is confined to the epidermis and strat corneum, whereas the various forms of hemoglobins are confined to vessel the dermis, and only indirectly exert any influence on optical radiation wit the overlying epidermis. Considering the absorption spectra and localizat of the major cutaneous pigments, and optical scattering for each layer, i possible to arrive at mathematical descriptions of cutaneous optics wh include most of the major variables in vivo. It must be emphasized, howev that a truly precise yet general quantitative model of cutaneous optics lacking.

STRUCTURE OF THE SKIN

A thorough review of the anatomy of the skin and its structures is n within the scope of this chapter (4–7); however, an overview of skin as a optical medium is presented here. Figure 1-1 of Chapter 1 is a diagrammat cross section of the skin showing three major tissue layers; the epidermis, t dermis, and the subcutaneous tissue. The thin outermost epidermis can b considered a sheet of epithelial cells called keratinocytes stacked 10–15 high These cells continually migrate outward from a germinative layer toward th surface. In the process, they flatten, die, and cement together to form proteinaceous, thin and tough outer layer called the stratum corneum. Th stratum corneum attenuates ultraviolet (UV) radiation before it reaches livin cells. The dermis is much thicker than the epidermis, has fewer cells, and i connective tissue. Blood vessels, lymphatics, and nerves course through th dermis. The deepest layer, the subcutaneous tissue, is mainly fatty tissue an acts as an insulator and a shock absorber.

Epidermis (Fig. 6-1)

The epidermal keratinocytes produce keratin, the fibrous protectiv lipoproteins of the skin. As keratinocytes differentiate and migrate outward they flatten, and granules appear within the cytoplasm, forming the stratu

are three major types of fibers present: collagen, reticulum, and elastin. Collagen is a long protein molecule woven into fibrils that allow stretching and contraction while maintaining tensile strength. Collagen makes up about 70% of the dry weight of the dermis, and is optically birefringent. The scattering of light by collagen is an important feature of the optics of the dermis. The finely branched reticulum fibers serve to link bundles of collagen together, and the scattered elastin fibers presumably lend an elastic quality to the dermis as a whole. Scattered cells called fibroblasts produce the fibers, proteins, and viscous materials of the dermis.

The uppermost dermal layer, the papillary dermis, contains an extensive plexus of capillaries, lymphatics, and nerves. Scattered lymphocytes leave the blood vessels of this layer, giving it more cellular appearance than the underlying reticular dermis. The reticular dermis is more fibrous, contains larger vessels, and has fewer cells and less ground substance than the papillary dermis. The dermis normally contains no melanin. The structure of skin is summarized in Table 6-1.

AN OVERVIEW OF SKIN OPTICS

The *transmittance* of a planar sample is defined as that fraction of the radiation incident upon one side of the sample that passes through and

Table 6-1. Skin Layers

Layer	Typical thickness, μm	Basic structure
Stratum corneum	8–15	Ten to 20 single-cell layers of densely packed, flattened, dead keratinocytes, in remarkably regular arrangement; rich in the lipoprotein keratin; contains melanin granules, carotens
Stratum granulosum	3	Granular cells between viable epidermis and stratum corneum; two to four cell layers
Stratum malpighii	50–150	Ten to 20 cell layers of keratinocytes, which produce the materials of the stratum corneum as they differentiate and become flattened, moving outward
Germinative layer	5–10	Single-cell layer of columnar basal cells, which divide to produce a continuing supply of keratinocytes; melanocytes also present, which produce melanin pigment granules and transfer these melanosomes into keratinocytes
Dermis	1000–4000	Connective tissue composed of collagen, reticulum, and elastin fibers and ground substance (gel); few cells compared to epidermis; outermost dermis (papillary dermis) contains many capillaries, lymphatics, and nerves

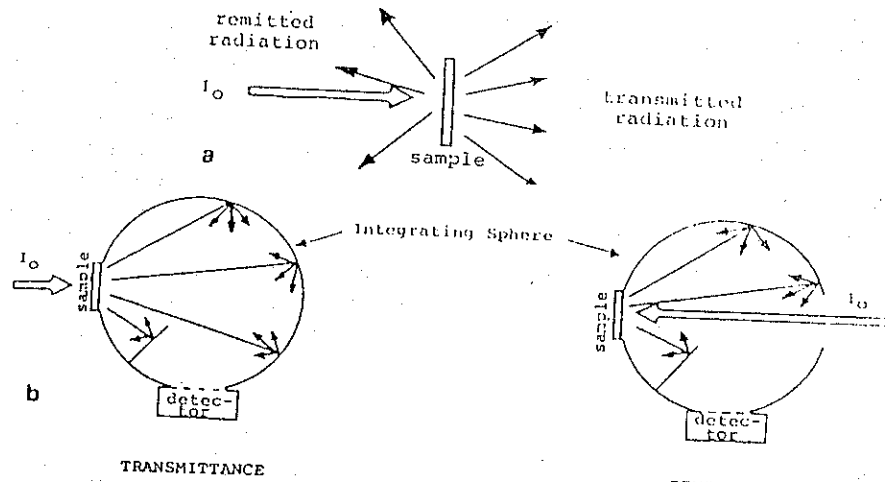


Fig. 6-2. (a) Transmittance is defined as (total transmitted radiation)/(incident radiation). (b) Measurement of transmittance (T) and remittance (R) of a sample, using an integrating sphere. The interior surface of the sphere is coated with $BaSO_4$ or MgO, which have nearly 100% diffuse remittance over the entire optical spectrum.

emerges from the other side of the sample. *Remittance* is defined as that fraction of the radiation incident upon one side of a sample that returns from or through the same side. The term "diffuse reflectance" is often used synonymously with remittance; however, the implication that the remitted radiation is truly diffuse (spatially isotropic) can sometimes be misleading. In order to measure the transmittance or remittance of skin layers, or of any turbid sample or sample with nonplanar surfaces, one must in general capture and measure all radiation entering each hemisphere on either side of the sample. This is most easily accomplished by using an integrating sphere, although other methods may also be used (1). These simple concepts and definitions are depicted in Fig. 6-2.

Initially, it is helpful to schematize the optics of normal skin as shown in Fig. 6-3. Upon encountering the skin at near-normal (nearly perpendicular) incidence, a small fraction of the incident radiation is reflected due to the step change in refractive index between air ($n_D = 1.00$) and stratum corneum ($n_D \approx 1.55$) (20). For normally incident radiation, this *regular reflectance* of an incident beam from normal skin is always between 4% and 7% over the entire spectrum from 250 to 3000 nm, for both Caucasoid and Negroid skin (1, 21). At larger angles of incidence, regular reflectance may reach higher values, as described by Fresnel's equations and shown graphically in Fig. 6-4 for a planar interface between air and a medium of refractive index 1.55. Although the skin surface is not strictly planar, the value of the regular reflectance from skin behaves similarly to that shown in Fig. 6-4 as the macroscopic angle of

spatially), polarized, and varies inversely with the fourth power of wavelength. The molecular scattering of sunlight by the sky is the most common example of this kind of scattering, called *Rayleigh scattering*. For particles with dimensions on the same order as the wavelength, scattering is many orders of magnitude stronger than Rayleigh scattering, more forward-directed, and, while varying inversely with wavelength, is not such a strong inverse function. Scattering of a beam of light by cigarette smoke is a common example of this type of scattering. When the particle size greatly exceeds the wavelength, scattering is highly forward-directed and essentially independent of wavelength (so-called Mie scattering). Clouds are a common example of large-particle scattering. They are white or grey because of the lack of wavelength dependence of scattering by their condensed water droplets, which are very large compared with the wavelengths of light. Red or orange clouds appearing at sunset are colored not because of scattering in the clouds, but because Rayleigh scattering along an increased atmospheric path has removed much of the blue, shorter wavelength radiation from the sunlight. Figure 6-5 shows the intensity of scattering by spherical particles of a given size and refractive index for these various situations.

Within the skin all of these general types of scattering occur, but, quantitatively, scattering by structures with dimensions on the order of optical wavelengths or somewhat larger must dominate over Rayleigh scattering. In particular, scattering by collagen fibers largely determines the penetration of optical radiation within the dermis (21).

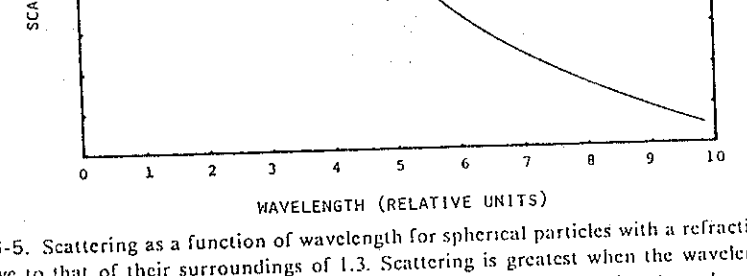


Fig. 6-5. Scattering as a function of wavelength for spherical particles with a refractive index relative to that of their surroundings of 1.3. Scattering is greatest when the wavelength and particle radius are approximately equal. The Rayleigh scattering region (wavelength much [illegible] scattering $\propto 1/\lambda^4$) extends infinitely beyond the right-hand side of the

In a nonscattering medium, absorption of monochromatic, collimated normally incident radiation follows the Lambert–Beer law, which states that the transmission of radiation varies inversely and exponentially with thickness of the medium and concentration of the absorber. The spectral transmittance of a thickness x of the medium is $T(\lambda) = 10^{-\epsilon(\lambda)cx}$, where $\epsilon(\lambda)$ is the molar extinction coefficient at wavelength λ, and c is the molar concentration of the absorbing chromophore. The absorbance of optical density (OD) of a sample is simply $-\log T(\lambda)$, or $\mathrm{OD} = \epsilon(\lambda)cx$. When scattering is also present, however, the effective pathlength of radiation within the medium is increased, which generally increases the likelihood of absorption, and in addition some radiation may be backscattered such that remittance also occurs. Thus, the measured optical density of a scattering sample containing a given amount of absorbing chromophore is always greater than that of a nonscattering but otherwise equivalent sample. The effect of scattering upon radiation transfer within a sample is dependent upon both the degree of scattering and the spatial distribution of the scattered radiation, as described above. Analysis of this situation, especially when a variety of absorbers and types of scattering are present, is highly complex.

If scattering is marked (a highly turbid medium), most photons experience multiple scattering before being absorbed or backscattered from the sample. In this case, the spatial distribution of the radiation as it passes through the sample quickly becomes isotropic (i.e., perfectly diffuse) regardless of the spatial distribution obtained for a single scattering. Furthermore, isotropic radiation cannot be "reorganized" to become nonisotropic by scattering of any type. If the radiation is isotropic, one can show that the average pathlength of photons along an infinitesimal path dx in any one direction is simply $2\,dx$ (23). For perfectly diffuse (isotropic) radiation the situation therefore becomes somewhat simplified, and one can derive absorption and scattering coefficients for diffuse radiation, equal to those for collimated radiation because of the pathlength argument given above, in terms of measurements of transmittance and remittance. The most popular model for this analysis is that derived by Kubelka and Munk (24–26), which is a special case of the general theory originally proposed by Schuster (27). An overview of this model, and its modification for application to skin, is presented below; the interested reader is referred to Kortüm (23), Wendlandt and Hecht (28), and Atkins (29) for exhaustive description of this and related radiation-transfer models. The differential (continuous) model proposed by Kubelka and Munk is certainly not the most elegant or thorough model of optical radiation transfer, but is simple and can be most readily applied to skin. In general, the more physically rigorous theories of radiation transfer require knowledge of structure and optical parameters which are difficult if not impossible to obtain for skin.

The Kubelka–Munk theory assumes that: (1) the sample has inhomogeneities causing scattering, which are small compared with the sa

In the Kubelka-Munk model, variations in the coefficients S and K are essentially independent of one another if the absorbing chromophores scatter weakly compared with surrounding nonabsorbing but strongly scattering structures. Fortunately, the structures of skin that lead to strong scattering, and hence determine S, are in general different than those chromophores present that determine K. For any given layer of skin, K is compositely determined by the concentration and distribution of those chromophores present. This is convenient because in normal skin certain chromophores, such as hemoglobins, bilirubin, and melanin, change rapidly, causing changes in absorption coefficients, whereas scattering coefficients should not change significantly until some gross alteration of structure occurs. As such, scattering coefficients are relatively constant compared with absorption coefficients, even between individuals. In particular, we shall use this basic model to describe radiation transfer within the dermis.

The Kubelka–Munk model also illustrates important concepts concerning the remittance and transmittance of samples with different relative degrees of absorption and scattering and of different thicknesses. In the case of a nonscattering sample, $S = 0$ and Eq. (3) reduces to give the Lambert–Beer law, stating that radiation traveling through a purely absorbing sample is attenuated as a simple exponential function of an absorption coefficient times the pathlength. When $K = 0$, there is no absorption and Eq. (5) reduces to $R + T = 1$, indicating that no radiation is lost. If a sample is infinitely thick, or simply thick enough that T approaches zero, one can rewrite Eq. (5) as

$$\frac{K}{S} = (R - 1)^2/2R \tag{9}$$

That is, the remittance of a thick sample depends solely upon the ratio of its absorption and scattering coeffients. This dependence is shown graphically in Fig. 6-7. Fortunately, the dermis is sufficiently thick that, for all optical wavelengths less than 600 nm, its transmittance is essentially zero. Therefore, for wavelengths less than 600 nm, the dermis can be thought of as a semi-infinite optical medium, greatly simplifying the analysis of skin remittance spectra as related to changes in dermal pigments. Finally, it is apparent that as the thickness of any particular sample decreases, R always decreases and T always increases. Indeed, it makes intuitive sense that, for a given turbid medium, very thin samples would transmit more and remit less radiation than thick samples. It shall be seen in the case of the stratum corneum, and to a large extent the entire normal human epidermis, that the layer is thin enough and has a high enough value of K/S that its direct contribution to remittance (other than the regular reflectance discussed above) can be essentially neglected over the entire visible and near-infrared spectral regions (21).

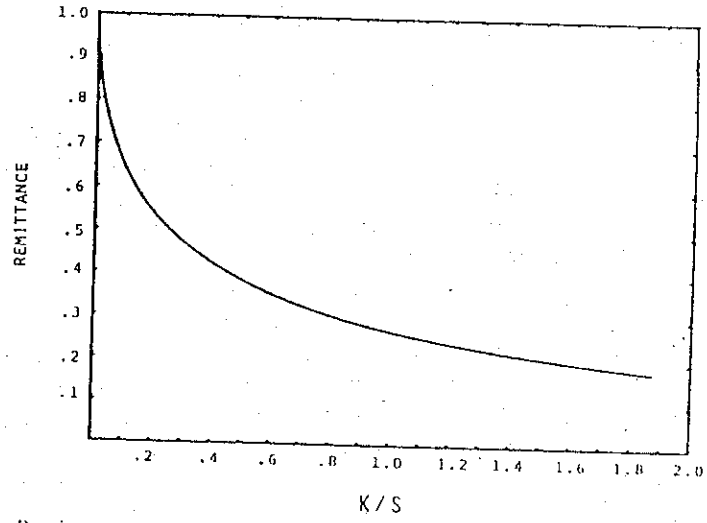


Fig. 6-7. Remittance of an infinitely thick sample as a function of K/S, according to the Kubelka–Munk theory. In practice, a sample may be considered infinitely thick whenever its transmittance is close to zero.

Optics of the Stratum Corneum and Epidermis

Many studies of the transmission of UV radiation through excised human epidermis and stratum corneum have been reported since the original work of Hasselbalch (30). Much of the early work, however, failed to account accurately for the diffuse nature of transmission through skin samples, largely because of inadequate instrumentation (30–36). Since the advent of commercially available spectrophotometers with integrating spheres, however, several groups have measured and published total transmission spectra of human epidermis (11, 37). Everett et al. (11) compared epidermal samples obtained by cantharadin blisters in vivo, by heat-and-stretch separation in vitro, and by suction in vivo. They also compared stratum corneum samples obtained from sunburn-induced vesicles (parakeratotic stratum corneum plus stratum granulosum) and by mild trypsinization of previously separated epidermis. As had been seen in previous studies, the UV-visible transmission of Caucasian stratum corneum or epidermis qualitatively resembles that of a solution of protein containing the aromatic amino acids tryptophan and tyrosine, with a minimum in transmittance near 275 nm due to absorption by these and other aromatic chromophores. Nucleic acids, with an absorption maximum near 260 nm, and numerous small aromatic molecules, especially urocanic acid, with an absorption maximum at 277 nm at pH 7.4, also contribute to the broad 275-nm absorption band seen in epidermis and stratum corneum. Finally, melanin content and distribution play a major role in

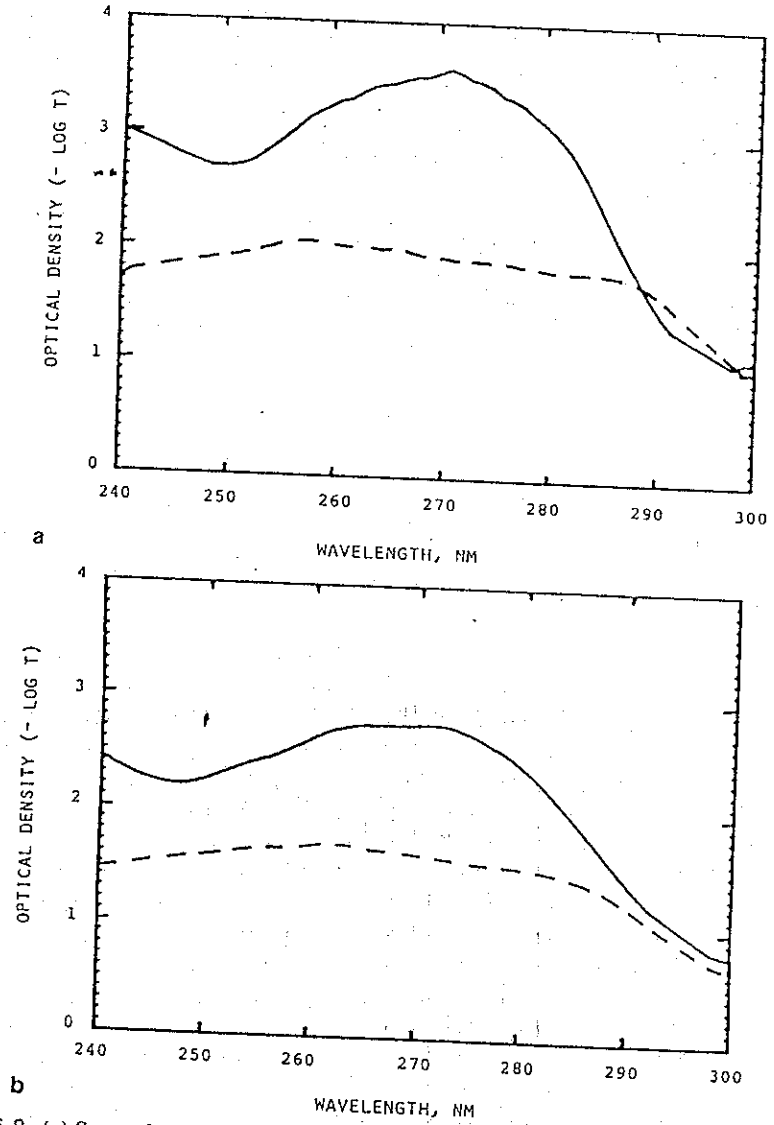


Fig. 6-9. (a) Spectral transmittance, expressed in optical density units, of Caucasian human epidermis sample taken with solar-blind PMT (—) vs. conventional broadband PMT (- - -) in the apparatus shown in Fig. 6-8. Note the large overestimation of transmittance which occurs due to tissue autofluorescence when a broadband detector is used. Sample was obtained from amputated thigh skin, was separated by immersion in 60°C water for 30 sec, and consisted of the entire epidermis minus the basal cell layer. (b) Spectral transmission, expressed in optical density units, of stratum corneum from skin adjacent to that shown in (a), taken with solar-blind PMT (—) vs. conventional broadband PMT (- - -). Sample was separated by 8-hr incubation at 37°C in the presence of 10 mg/ml staphylococcal scalded-skin syndrome epidermolytic toxin in Hepe's buffer with 20% FCS, and consisted of stratum corneum plus stratum granulosum.

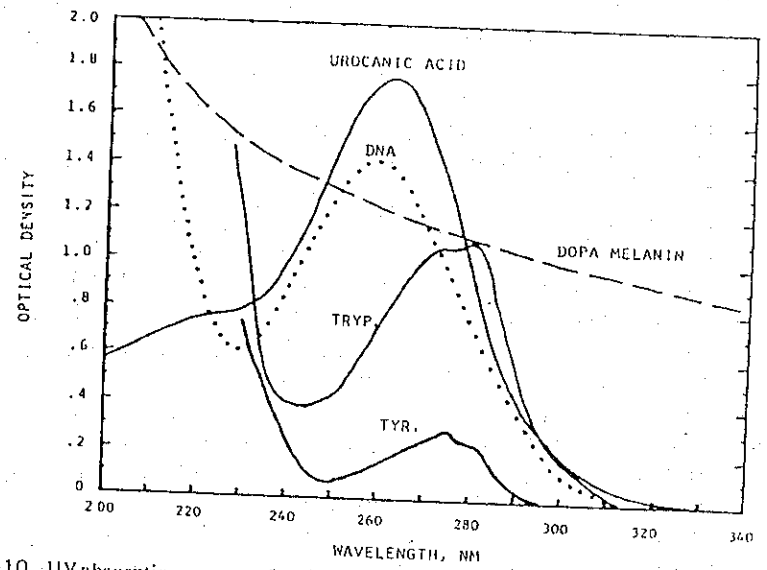


Fig. 6-10. UV absorption spectra of major epidermal chromophores. DOPA-melanin, 1.5 mg% in H_2O; urocanic acid, 10^{-4} M in H_2O; calf thymus DNA, 10 mg% in H_2O (pH 4.5); tryptophane, 2×10^{-4} M (pH 7); tyrosine, 2×10^{-4} M (pH 7). The broad epidermal absorption band near 275 nm is the result of absorption by protein, urocanic acid, nucleic acids, and other aromatic chromophores.

near-UV and visible transmittance of the epidermis than do variations in thickness. Although he used rather crude methods for measuring transmittance, and did not consider that there might be racial differences in the density of the stratum corneum as has been subsequently suggested (46), his basic conclusion is beyond dispute. Indeed, it is common experience that variations in melanin pigmentation have profound effects on the transmittance of solar UVB into and through the human epidermis to cause sunburn. In the visible portion of the spectrum, melanin is essentially the only pigment affecting the transmittance of normal human epidermis, giving rise to the wide range of discernible skin colors from "black" to "white." The 300-nm transmittance of full-thickness epidermis including the basal cell layer varies by 2–3 orders of magnitude from fair-skinned to darkly pigmented individuals (R. R. Anderson, unpublished observations). The relative importance of melanogenesis vs. epidermal hyperplasia as two UV-inducible mechanisms for photoprotection against various wavelength regions can be seen from the absorption spectra given above. For UVC and UVB wavelengths, even mild hyperplasia alone could exert a highly significant photoprotective effect because of the absorption by protein and other constitutive epidermal chromophores. For protection against UVA or visible radiation effects, however, hyperplasia alone would not offer practically significant photo-

spectrophotometrically. Whereas there was a fivefold average difference in the UVB and UVA transmittance of black vs. white epidermis, the average racial difference in UVB and UVA transmittance of the stratum corneum samples was less than twofold. This study therefore suggests that the large racial differences in sensitivity to UV radiation of 10–30-fold (54, 55) correlate poorly with the small racial differences noted in corneal transmission. However, the minimal erythema dose of black and white subjects has never been directly compared with accurate corneal or epidermal transmittance measurements of skin samples from the same subjects. Such a study might be a rather direct means of determining the tissue layers involved in initiating delayed erythema response.

Urocanic acid is thought to play some role as an "endogenous sunscreen" of the epidermis and stratum corneum (56, 57). Unlike the dermis and most other tissues, the epidermis lacks urocanase (58), the enzyme that oxidatively degrades urocanic acid, while histidine deaminase, the enzyme responsible for urocanic acid synthesis, is present and apparently most active in the latter stages of keratinocyte differentiation at the stratum granulosum (59). There is some evidence that human epidermal urocanic acid levels (44) and histidine deaminase activity in guinea pigs (58) increase for weeks following UV radiation exposure, but these observations may be wholly or in part due to UV-induced epidermal hyperplasia (45).

In those studies that have compared diffuse vs. direct (total transmittance vs. transmittance along an optical path in line with the incident beam) transmittance of epidermis or stratum corneum, the ratio of diffuse/direct transmission does not appear to be wavelength dependent (11, 34, 35) for either UV or visible wavelengths. This broadband independence of wavelength suggests that the diffuse nature of epidermal transmission of UV wavelengths is due more to the irregular refractive surface of skin than to particle scattering within the epidermis. Furthermore, less than 5% of collimated, normally incident radiation in the 280–3000-nm region is remitted by scattering within Caucasian epidermis (21). This observation is consistent with a thin sample in which backscattering (S) is small compared with the reciprocal of sample thickness ($\simeq 100\ cm^{-1}$), and/or absorption relative to scattering (K/S) is large. The epidermal transmittance spectra presented above for fair-skinned Caucasians indicate that most of incident near-UV, visible, and near-infrared radiation is transmitted through epidermis. Scattering of visible light within the epidermis is minor enough that, when a layer of oil is applied to diminish refraction at the irregular skin–air interface, one can readily see the capillary tufts of the dermis upon microscopic examination (60). The logical conclusion from the above observations is that whatever backscattering occurs in normal epidermis over this spectral region is for practical purposes weak, and that any strong scattering within epidermis that does occur must be highly forward-directed, i.e., off-axis refraction occurring

For wavelengths less than 320 nm, epidermal absorption increases dramatically. Therefore, even though one would expect these shorter wavelengths to experience greater scattering, a concomitant large increase in absorption, and hence K/S, explains why little incident energy in this region is remitted (cf., Fig. 6-7). If one neglects epidermal backscattering over much of the optical spectrum, a greatly simplified analysis of in vivo skin remittance spectra can be obtained, as shall be discussed. However, despite its central role in cutaneous and systemic photoprotection, a precise model of epidermal optics is still lacking.

Optics of the Dermis

Relatively few studies of optical radiation transfer in the dermis or other connective tissue have been reported. Hardy et al. (12) performed goniometric studies (1) of transmission and remission of a collimated incident beam through tangentially sectioned Caucasian and Negroid skin samples of various thicknesses and including both the epidermis and various amounts of dermis. Their findings were that, as greater thicknesses of dermis were included, the transmission decreased with thickness and was more diffuse, suggesting multiple scattering. The Lambert–Beer law was invalid for the visible and near-infrared wavelengths tested as the thickness became greater than 0.5 mm, suggesting that considerable scattering was present in the dermis. In general, longer wavelengths across the visible and near-infrared spectra exhibited both greater and more forward-directed (less diffuse) transmission, which is consistent with scattering by structures with dimensions on the same order as, or greater than, the wavelength of light, most probably bundles of collagen. Of the wavelengths studied, Hardy et al. found 1.23 μm (1230 nm) to be the most penetrating wavelength.

Findlay (61), in an attempt to explain blue skin colors, noted that when the epidermis was removed from a blue nevus of Ota (caused by an abnormal deposition of melanin in the dermis), the dermis was "intensively blue upon direct inspection." He correctly concluded that the epidermis had little to do with blue skin, and went on to measure visible transmittance and remittance spectra of thin sections of pig dermis, or sheets of dura mater (a thin sheet of connective tissue) of newborn humans. Both showed greater transmittance at longer wavelengths, similar to Hardy et al.'s findings, but exhibited greater remittance of shorter wavelengths. Summing Findlay's transmittance and remittance spectra gives values close to 1.0 (100%) across the entire visible spectrum, indicating that very little visible light is absorbed by dura mater or pig dermis in vitro. Visualizing the dermal deposition of melanin pigment in the nevus as being the optical equivalent of providing only a thin layer of dermis for remittance of incident light, Findlay concluded that "subtractive color mixing" accounted for blue skin colors. The only explanation for the spectral transmittance and remittance curves is that light scattering in the

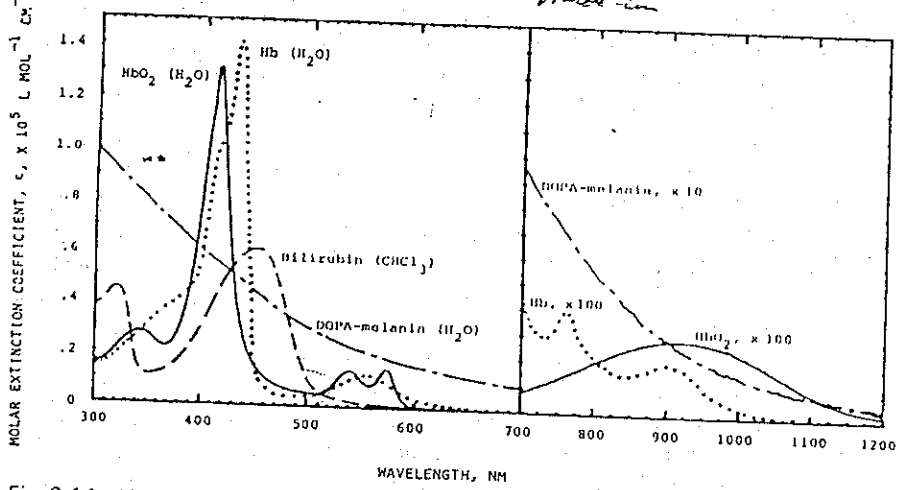


Fig. 6-14. Absorption spectra of major visible-light-absorbing pigments of human skin, HbO_2 (—), Hb (· · ·), bilirubin (- - -), and DOPA-melanin (- · -). Parentheses indicate solvent. The spectrum shown for DOPA-melanin is the absorbance on a scale of 0 to 1.5 of a 1.5 mg % aqueous solution. Not shown is beta-carotene, which has a broad absorption band qualitatively similar to that for bilirubin in the 400–500-nm region, with maxima at 466 and 497 nm in $CHCl_3$. Note scale changes in the near-infrared.

oxyhemoglobin is not nearly as great as if these chromophores were uniformly distributed throughout the dermal tissue. This effect is conceptually similar to the difference in optical absorption between a sheet of paper with numerous small dots of ink on it and a similar sheet with the same quantity of ink uniformly coating the paper. Second, since the penetration of optical radiation in the tissue is wavelength dependent, stratification of pigments at

Table 6-2. Approximate Depth for Penetration of Optical Radiation in Fair Caucasian Skin to a Value of $1/e$ (37%) of the Incident Energy Density

Wavelength, nm	Depth, μm
250	2
280	1.5
300	6
350	60
400	90
450	150
500	230
600	550
700	750
800	1200
1000	1600
1200	2200

different depths influences the spectral distribution of the radiation reach
given stratum. For example, the blue visible light corresponding to the m
absorption band of beta-carotene is essentially absent at the 1–4-mm de
associated with the upper limits of the subcutaneous fat layer. The ye
color of carotenemic individuals can therefore be due only to that b
carotene in the stratum corneum, epidermis, and superficial dermis.
otenemic individuals typically exhibit selective yellowing of the palms
soles, sites in which the thick corneum can retain enough carotenoids
noticeably affect skin color. Similarly, the stratification of the cutane
vascular architecture from tiny capillaries to venules, arterioles, and ma
vessels at greater depths influences optical absorption by cutaneous blo
Only the superficial vessels—capillaries and the venular plexis—will
exposed to significant blue or UV radiation.

Because of the increased dermal scattering and optical absorption in vi
for wavelengths less than 600 nm, and the high optical absorption
wavelengths longer than about 1300 nm by water, an optical "window" exi
in skin and most other soft tissue in the region 600–1300 nm. Another su
"window" exists from 1600 to 1850 nm, between two water absorption ban
Whenever it is possible to use some portion of the penetrating 600–1300-n
wavelength region to cause phototoxicity, the volume and depth of tissu
affected will be large. This has been taken advantage of in hematoporphyri
derivative (HPD) photoradiation therapy for cancer, using 630-nm radiatio
(see Chapter 23). The 600–1300-nm region carries a quantum energy of onl
2–0.95 ev, respectively, and hence can only exert photobiologic influence
other than heating by affecting very weak bonds. For example, the productio
of singlet oxygen, the putative intermediate involved in HPD phototoxicity
requires approximately 1 eV. It is possible that other photodynamic
photosensitizers exist which can produce singlet oxygen upon absorption o
wavelengths even longer than the 630-nm (1.97 eV) radiation used with HPD.

Some weak endogenously occurring bonds are also influenced by 600–
1300-nm radiation, and it is therefore possible that these long wavelengths
have some effect on metabolic processes in skin and perhaps even on internal
organs. CO and O_2 liganded to hemoproteins can often be photodissociated
from the heme groups. Carbon monoxide bound to hemoglobin, myoglobin,
and some cytochromes has been shown to be photodissociable (64–66), and
the action spectrum for photodissociation of human HbCO includes wave-
lengths as long as 700 nm (67). Despite earlier, largely uncontrolled
experiments suggesting an effect of visible light on clearance of CO from
animals (68, 69), deliberate exposure to intense radiation in the 400–1000-nm
region, even directly upon the lungs, has little effect upon clearance of CO
from experimentally CO-poisoned animals (67). It is nonetheless possible that
visible and near-infrared wavelengths might influence cutaneous blood gas
exchange, oxidative phosphorylation, or other metabolic processes. Pre-
liminary measurements of spectral transmission through the human chest wall

but T_e and R_D are still treatable as separate parameters (21). An improvement in this simple model would be to model both the epidermis and dermis as discrete layers using the Kubelka-Munk or similar theory.

The main advantage of this model is its simplicity. By separating epidermal and dermal elements, the effect of dermal chromophores upon the dermal remittance parameter R_D might be treated using the Kubelka–Munk or similar radiation transfer theories. The dermal scattering coefficient S should not be greatly affected by changes in states of vasodilatation in the papillary dermis, because (1) S is determined largely by scattering by collagen; (2) blood vessels occupy a relatively small volume fraction of the dermis; and (3) the analogous scattering coefficient for whole human blood is only slightly less than that determined for bloodless dermis in vitro (R. R. Anderson; unpublished observations). The effects of edema on S may be more drastic, but are unknown at present. Treating S as a constant, then, one might use variations in R_D caused by dermal chromophores to calculate K, the effective dermal absorption coefficient corresponding to "concentrations" of dermal chromophores.

For the model to be useful practically, one must be able to somehow measure the melanin-dependent epidermal transmittance term T_e independently of the effects of dermal chromophores. Fortunately, there are two widely separated spectral regions in which melanin absorbs, but none of the normal dermal chromophores exerts a major influence on in vivo remittance. One such region is 650–700 nm, where blood shows very little absorption, the other is 330–400 nm, where melanin exhibits high absorption but other epidermal chromophores do not (Fig. 6-10), absorption by hemoglobin or oxyhemoglobin is relatively weak (Fig. 6-14), and the dermal penetration depth is superficial (Table 6-2) such that only the small volume of blood present in superficial capillaries can significantly affect the remittance.

Remittance measurements in these two spectral regions have been used for monitoring melanin pigmentation, with the choice of wavelength based largely upon empirical observations rather than on an optical model for in vivo spectral remittance. Numerous authors simply have used a single measurement of skin remittance in the red visible region for this purpose (13, 71, 74, 75). The technique is adequate for measuring gross pigmentation differences across the wide "spectrum" from Caucasians to blacks, but is not especially sensitive for detecting small differences in melanin pigmentation of lightly pigmented skin, because melanin's absorbance is not particularly high in the red visible region. In contrast, remittance in the spectral region around 350 nm is quite sensitive to small differences in the pigmentation of fair-skinned individuals. Consequently, UVA (320–400 nm) photography of skin (76) or the visible autofluorescence of skin excited by UVA (77) can be used to visualize small variations in melanin pigmentation.

The measurement of erythema has most often been accomplished by

comparing remittance in the 540–575-nm (green) region with remittance in 630–700-nm (red) region (74, 75, 78, 79). Neither absorption by melanin the dermal scattering coefficient varies extremely between these two nea wavelength regions, but absorption by oxyhemoglobin is several orders magnitude higher in the wavelength region from 540 to 575 nm than from to 700 nm. Most authors have chosen to arithmetically subtract the gr remittance from the red remittance to provide a scale for monitor vasodilatation. Despite the arbitrary nature of such analysis, this sim approach does offer a more objective means than visual inspection monitoring vasodilatation. However, in view of the basic model present above, arithmetic subtraction of the remittance leads at best to an arbitra nonlinear scale for vasodilatation which is also somewhat dependent up melanin pigmentation.

Expressing the spectral remittance of skin in optical density un ($OD_R = -\log R$) and in essence subtracting these values at the gre wavelengths associated with HbO_2 absorption from those in the red regio gives better results. Here, one must be aware that OD_R is an *apparent* optic density unit, and will be proportional to K only as a first approximation, ov narrow spectral regions where S can be treated as wavelength independen This approach was taken by Feather et al. (80), who used spectral comparison of OD_R between UV-exposed and adjacent unexposed sites to establish scale for melanin pigmentation and erythema. Melanin pigmentation was mea sured by determining the slope for differences in OD_R between exposed an unexposed sites over the region 645–705 nm, based on the rationale tha melanin is the major cutaneous pigment affecting remittance in this region and that melanin's absorption spectrum climbs gradually toward the shorte wavelengths. An index of vasodilatation was defined by taking the area unde the OD_R curve corresponding to the 542-nm and 577-nm absorption bands o oxyhemoglobin, i.e., in the range 510–610 nm. These scales for erythema an pigmentation experimentally proved not to be entirely independent, and the authors derived a linear correction coefficient, which, when applied to th OD_R measurement scale for melanin, rendered their vasodilatation scal relatively independent of melanin pigmentation.

This study offers significant improvement over previous methods because optical density units are used to derive measures of melani pigmentation and vasodilatation; the measures for pigments should therefor show greater linearity with actual changes in melanin "concentration" o blood volume. However, the effect of variations in absorption coefficients (pigments) upon the effective pathlength and depth being sampled was no included in the analysis. Furthermore, the analysis is comparative betwee sites and it is not clear whether absolute states of vasodilatation o pigmentation can be assessed in this manner.

Application of more rigorous radiation-transfer models for the purpos

over the dermis, the fluorescence increases and is subjectively similar to that for the full-thickness tissue before separation and for in vivo skin (R. R. Anderson, unpublished observations). These observations strongly suggest that fluorescence seen under Wood's lamp is largely due to dermal autofluorescence, caused by UVA penetrating the epidermis, and of course viewed through the epidermis. The fact that variations in epidermal pigmentation are more apparent under Wood's lamp than under visible light is therefore most likely due to the greater absorption by melanin in the UVA vs. the visible spectrum. When the melanin is dermal, however, a very different effect is obtained. UVA penetrates the dermis only superficially compared with visible light, largely because of dermal scattering (cf. Table 6-2). Thus, most of the dermal autofluorescence seen under Wood's lamp is limited to the superficial dermis, and melanin pigment occurring beyond some shallow depth (on the order of 50 μm) within the dermis will cause little or no reduction in this autofluorescence. The effect is conceptually similar to that discussed above for blue skin colors when dermally deposited melanin is present, but is more pronounced under Wood's lamp because UVA is of even shorter wavelength than blue light. The fluorophores or phosphores responsible for visible autofluorescence of the dermis are unknown.

PHOTOMEDICAL TREATMENTS AND CUTANEOUS OPTICS

Knowledge of cutaneous optics is helpful both in explaining certain photobiologic phenomena and in designing new forms of phototherapy or photochemotherapy. In the absence of spectrally dependent tissue optics, biologic action spectra parallel absorption or excitation spectra for the chromophores initiating a biologic response pathway. In skin, however, action spectra will only resemble absorption spectra for a putative chromophore if one corrects for the spectral attenuation of incident radiation by the tissue before reaching the chromophore. As such, the optical properties of the tissue overlying the chromophore and the depth of the chromophore become very important in determining the relative effectiveness of incident radiation as a function of wavelength.

For example, the distinct minimum near 280 nm in delayed erythema action spectra of human skin is largely if not entirely due to absorption of incident radiation of the stratum corneum and epidermis (88). It recently has been shown that the action spectrum for phototherapy of psoriasis vulgaris is similar to that for delayed erythema in normal skin, except for wavelengths less than approximately 295 nm, which are comparatively ineffective (89) (cf. Chapter 17). Wavelengths of 295 nm are on the edge of the strong absorption band for stratum corneum centered near 275 nm. Therefore, the most direct explanation for this important observation is that transmission of wave-

lengths less than 295 nm through the thick stratum corneum of pso
plaques is much less than that for normal skin, resulting in a gross differer
the phototoxicity between psoriatic and normal skin induced by
wavelengths. The 275-nm apparent absorbance (−log T) or normal,
skinned Caucasian stratum corneum is typically 1.5–2. The psoriatic stra
corneum, being several times thicker, may have an absorbance of 4–6 at
wavelength. The net result would be several orders of magnitude differen
the incident dose at 275 nm necessary to cause phototoxic effects in vi
keratinocytes of psoriatic vs. normal skin. At the longer UV wavelen
outside this broad absorption band, i.e., greater than or equal to appr
mately 300 nm, the effect of the increased corneal thickness in plaques wo
be much less dramatic. Other explanations, such as different mechani
initiated by different chromophores at different sites being involved in dela
erythema of normal skin vs. regression of psoriatic plaques, are also possi

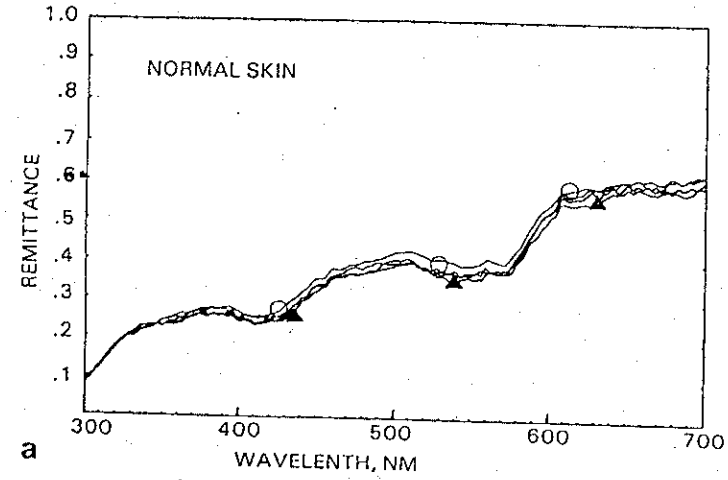
The depth to which optical radiation penetrates cutaneous tissues va
by several orders of magnitude over the 250–1300-nm spectral region. Beca
many phototoxic drugs exhibit wide action spectra, it is possible in theory
control the depth of photochemotherapeutic treatments of skin by sim
varying the wavelength. For example, if the phototoxic action spectr
includes most of the visible spectrum, the depth of treatment can be control
to either maximize or avoid exposure of blood cells. It is likely that the dep
of photochemotherapeutic treatments with HPD or other phototoxic dru
can be controlled in this manner not only for skin, but also for oth
connective tissues or organs accessible by optical fiber endoscopes.

MANIPULATING THE OPTICS OF SKIN

Decreasing Photobiologic Sensitivity

The sensitivity of skin to UV radiation can be decreased or increased b
topical applications. If a UV-absorbing or -scattering compound is applied
the skin in the form of a "sunscreen," sensitivity to radiation in the spectra
regions involved will decrease. Numerous sunscreen formulations exist, wit
different absorption spectra, water solubilities, and other characteristics (c
Chapter 15).

The relative decrease in sensitivity as measured by increases in the
minimal delayed erythema dose (MED) at specified wavelengths is propor
tional to the relative decrease in transmittance of the stratum corneum after
application of the sunscreen (90). Calculations of protection offered by any
given sunscreen on the basis of assuming a uniform-thickness layer of the
sunscreen with a known absorption coefficient as measured in solution give
falsely high estimates of protection (91). The skin surface is far from planar



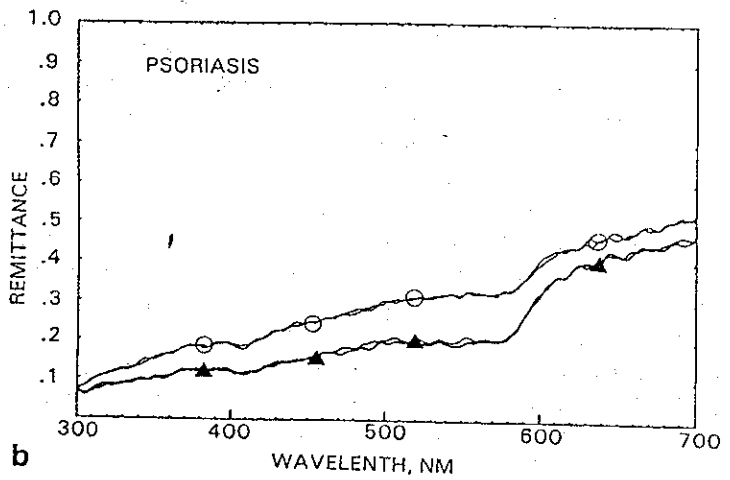


Fig. 6-16. (a) Spectral remittance of normal Caucasian skin before (O) and after (▲) application of mineral oil. (b) Spectral remittance of typical psoriasis vulgaris plaque before (O) and after (▲) application of mineral oil. The immediate, broad-spectrum decrease in remittance is consistent with a reduction of regular reflectance occurring at the surface(s) of the plaque.

Because the optics of normal skin is not significantly affected by application of oils, one can easily maximize transmission of UVB or UVA radiation into psoriatic plaques during phototherapy without affecting the responses of normal skin, which is generally the limiting factor in the aggressiveness of therapy. Oily lubricants, such as petrolatum, when applied before UVB treatment of psoriasis, significantly enhance phototherapeutic effectiveness (96). Some or all of this effect must be related to the optical changes discussed above. For FS40-lamp UVB phototherapy, the addition of tar compounds to an oily base may be no more effective than the base alone

(96). Because of the very wide spectral character of the reduction in regul reflectance of plaques, use of oily lubricants should be of some benefit for wavebands used in phototherapy or photochemotherapy of psoriasis. It probable, however, that the added therapeutic benefit will be somewh greater for relatively collimated incident radiation sources at wavelengths le than or equal to approximately 300 nm, which are more strongly absorbed stratum corneum.

LASER-INDUCED SELECTIVE THERMAL DESTRUCTION OF PIGMENTED STRUCTURES

Lasers are sources of optical radiation with the special characteristics essentially absolute monochromaticity, spatial and temporal coherence, and for many lasers, high power. The coherence of lasers allows their output beam to be focused to very small diameters, on the order of the wavelength of th radiation emitted. Thus, it is possible to achieve extremely high optical energy densities over areas small enough to destroy, for example, only a smal fragment of a single chromosome (97). Pulsed lasers often exhibit extremely short pulse times, as short as 10^{-12} sec or less, and as long as tens o milliseconds, and peak power outputs as high as 10^{10} W or greater. These characteristics of pulsed lasers have made it possible to study very fast processes, such as time-resolved spectroscopy of the transient excited states involved in photochemical reactions. Continuous (CW or nonpulsed) lasers are also available. The many different common types of lasers emit radiation over the spectrum from the vacuum-UV through far-infrared, usually only in one or more discrete spectral lines for each type of laser. Pulsed or continuous tunable organic dye lasers are also available, in which the output wavelength can be tuned over the near-UV through near-infrared spectral regions. In addition, special crystals for the nonlinear generation of optical harmonic frequencies can be used with pulsed lasers to shift the output wavelength to precisely one-half or one-third of the fundamental wavelength.

Medically, lasers have in general, but not exclusively, been used for surgical applications. The vast majority of optical energy at any wavelength absorbed in tissues is rapidly converted to heat by nonradiative processes. The high focal power of lasers can therefore cause confined heating and thermal destruction of tissue, depending upon the wavelength, power, and beam size of the laser, and the optical absorption and scattering properties of the tissue. The main advantage of using a laser instead of a surgical scalpel is that blood vessels are thermally coagulated, reducing or eliminating blood loss. Also, if the power and exposure duration of the laser are carefully controlled, the depth of the incision and photocoagulation can be controlled with some precision. The most common "surgical knife" laser is the continuous CO_2

Table 6-3. Estimated Times for Vessel Temperature to Drop to $1/e$ (37%) of Initial Temperature As a Function of Vessel Diameter, Assuming No Blood Flow (101)

Vessel diameter, μm	Estimated thermal relaxation time, msec
5	0.01
10	0.05
20	0.2
30	0.4
50	1.2
70	2.4
100	4.8
150	11.0

between the target structure and its surroundings will be achieved. When the incident energy ceases, this localized heat in the target structure will then diffuse to surrounding structures, and in the process be dissipated over a greater volume such that the macroscopic temperature rise of the tissue will be much less than that achieved briefly in the target structure. The time associated with retention of heat within a target structure depends solely upon the size and thermal properties of the structure and its surroundings. For blood vessels, removal of heat by blood flow must also be considered.

Assuming that blood, blood vessels, and the dermis have approximately the same thermal properties as stagnant water, and modeling vessels as infinite cylinders, one can apply thermal diffusion theory to estimate the time for retention of heat in a vessel that is instantaneously heated to some temperature greater than that of the surrounding tissue. Table 6-3 shows estimated times for the vessel temperature to drop to $1/e$ (37%) of its initial temperature as a function of vessel diameter, assuming no blood flow (101). For the range of ectatic vessel diameters encountered in port-wine lesions the estimated thermal relaxation times range from about 0.5 to about 15 msec. Assuming a blood flow velocity of 0.5 cm sec^{-1} or less, the blood will move, at most, a few tens of micrometers during the longest thermal relaxation times. Given that the length of the vessel exposed to significant laser radiation is much larger than this distance, one can, for the purposes of estimation, neglect the influence that axial removal of heat by blood flow may have upon the thermal relaxation time. Therefore, if the exposure duration of the laser is kept to approximately 1 msec or less, maximum differential heating of blood vessels should occur.

Finally, one can estimate the transient microscopic (target structure) and macroscopic temperature rises produced as a function of incident energy density if the optical and thermal properties of the tissue are known. Based on

the optical data and models given above for normal fair-skinned Caucasian it is reasonable to expect that the average energy density at 577 nm within t first 200 μm of the dermis is roughly 50% of the incident energy density. If t exposure duration is less than 1 msec, the peak vessel temperature rise will simply the energy absorbed per unit vessel length divided by the product vessel cross-sectional area and the heat capacity of blood. For a 50-μm diameter vessel and an initial ambient skin temperature of 33° C, the estimate peak vessel temperature is 58° C for an incident energy density of 2.5 J cm 80° C for 5 J cm^{-2}, and 130° C (vaporization) for 10 J cm^{-2}. The estimated pea temperatures for vessels of 20 and 100 μm diameter are similar. Therma denaturation of proteins typically occurs over the 60–100° C temperatur range, such that one would expect an incident energy density on the order of J cm^{-2} to produce significant thermal damage to vessels, while incident energ densities greater than or equal to approximately 10 J cm^{-2} might caus vaporization of blood within the vessel.

Preliminary investigations in normal Caucasian skin (102) using a pulsed organic dye laser operating at 577 nm with a pulse duration of 0.3 μsec, which is much less than the calculated thermal relaxation times for even the smalles vessels, support the above theoretical estimates. In normal fair-skinned Caucasians, exposures of 3–5 J cm^{-2} incident energy density from this laser immediately cause purpura, with no signs of gross damage to the epidermis. Histologically, sites exposed to 3–5 J cm^{-2} incident energy density show marked vasculitis, and some hemorrhaging when biopsied immediately after exposure, and subsequently a polymorphonuclear cell infiltrate. Histologically, the epidermis appeared normal. For incident energy densities of 10 J cm^{-2} or greater, purpura and occasionally epidermal perforation occurred. Histologically, a subepidermal blister and/or rupture of the epidermis were apparent for incident doses of 10 J cm^{-2} or greater. These observations are consistent with vaporization damage. When the laser output was tuned to 600 nm, which is not strongly absorbed by either blood or melanin, no effects were noted up to an incident energy density of 20 J cm^{-2}; higher exposure doses were not possible at this wavelength.

Whether organic dye lasers operating at 577 nm offer a better treatment for port-wine stains is unknown as of the printing of this chapter. However, the theoretical considerations given above have been substantiated, and a conceptually similar approach to predicting laser-induced selective thermal damage might be applied for other medical applications of lasers.

SUMMARY

The optics of human skin is dynamic and variable, and depends upon many different chromophores and scattering structures. Nonetheless, a general and quantitative approach to modeling cutaneous optics is possible,

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