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7

The Photochemistry and Photobiology of Vitamin D_3

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SUNLIGHT AND ITS CURATIVE EFFECTS ON RICKETS

Some historians state that the disease rickets was reported to occur in humans as early as the second century A.D. (1, 2), but the disease was not considered a significant health problem until people began to congregate in the cities of Northern Europe during the Renaissance (1–4). In the mid-17th century, Whistler, DeBoot, and Glisson each independently recognized many of the major diagnostic signs of this disease and established rickets as a clinical entity. They noted that it was associated with deformities of the skeleton, particularly enlargement of the epiphyses at the joints of the long bones and the rib cage (rachitic rosary), enlargement of the head, bending of the spine, curvature of the thighs, and flabby and toneless legs that were usually unable to sustain the weight of the body (1, 3, 4). The incidence of this debilitating bone disease increased dramatically during the industrial revolution, especially in Northern Europe and North America, and, by the latter part of the

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which aids in understanding photobiologic phenomena, in making useful optical measurements of cutaneous and systemic conditions and responses, and in improving and devising new photomedical treatments. By deliberately changing the optical properties of skin and selecting appropriate wavelengths lying within action spectra for photosensitized responses, one can often maximize therapeutic effectiveness, minimize risks to normal skin, and determine the depth of tissues treated by photochemically induced mechanisms. For thermally induced mechanisms, one can similarly choose optimal wavelengths and exposure durations for achieving selective thermal damage to pigmented target structures. There remain many questions regarding the optics of human skin and other organs accessible to optical radiation, and probably a greater number of undiscovered photomedical applications which await this knowledge.

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laser, operating at a wavelength of 10.6 m (10,600 nm). Water absorbs very strongly at this wavelength, and therefore the CO_2 laser generally causes relatively equal thermal destruction of many different soft tissues for a given set of exposure conditions. In contrast, the thermal damage and volume or depth of tissue or structures affected by lasers operating over the near-UV, visible, and near-infrared spectral regions vary greatly depending upon the optical properties of the specific tissue exposed.

The laser most commonly used for dermatologic treatments of port-wine stains, hemangiomas, and tattoos is the argon-ion laser, a continuous laser emitting 1–20 W total power in emission lines at 476, 488, and 514 nm. Pulsed ruby lasers, emitting pulses of a few milliseconds' duration at 694 nm, have also been used for treatment of tattoos and pigmented lesions (98). Choice of lasers and exposure parameters has in general been based upon clinical comparison of results with various lasers rather than an understanding of the mechanisms for treatment, the optical and thermal properties of the tissue, and a rational theoretical approach to maximize the therapeutic benefits and minimize adverse reactions such as scarring. Still, argon laser treatment of port-wine stains and other cutaneous hemangiomas or telangiectases has been developed to the extent that it is often the treatment of choice.

Both grossly and histologically, argon-laser treatments of port-wine stains as currently performed cause extensive thermal necrosis of the entire epidermis and the first 1–2 nm of dermis (99). The lesion is blanched immediately, and a superficial crust of thermally denatured tissue is formed. Over 3–6 months following treatment, fibrosis and reepithelialization occur. The characteristic numerous ectactic vessels responsible for the pink or red color of port-wine stains are replaced by smaller vessels, and a more normal skin color results. Patients under the age of approximately 30 tend to exhibit pink-colored lesions in which the ectatic vessels are of smaller diameter than in older patients, whose lesions tend to be red or violaceous. The pink lesions associated with younger patients generally respond poorly to treatment (100). Often, superficial or, occasionally, extensive scarring results, and because the epidermis is destroyed, there is some risk of infection and loss of normal epidermal pigmentation. Some denaturation of collagen and fibrosis may in fact be necessary for the formation of smaller vessels after treatment, but it is unlikely that extensive fibrosis or epidermal necrosis is necessary for successful treatment.

Through appropriate theoretical consideration of the optical and thermal transfer processes involved in causing thermal damage, it has been possible to select laser wavelengths, exposure times, and incident energy densities which cause highly selective thermal damage to vessels without significant damage to other dermal structures or epidermis (R. R. Anderson and J. A. Parrish, unpublished observations). The general theoretical approach used is applicable to any situation in which one wishes to selectively

damage a particular pigmented structure in skin or other organs, and may therefore find application for many other treatments as well.

If the overall goal is to selectively heat a certain pigmented structure such as vessels, the wavelength chosen should correspond to high absorption by the targeted structure relative to other optically absorbing structures. Choice of wavelength also determines the depth to which the optical radiation will penetrate with sufficient optical energy density to cause thermal effects. For vessels, oxyhemoglobin is the logical target chromophore, because it is entirely intravascular and is the dominant hemoglobin species. The wavelengths suitable for consideration are the HbO_2 Soret absorption band at 418 nm and the α and β absorption bands at 542 and 577 nm. Despite the higher extinction coefficient of the Soret band, this wavelength can be rejected on the grounds that penetration into the dermis (cf. Table 6-2) is insufficient for exposure of vessels at depths greater than approximately 0.1 mm. Furthermore, absorption by epidermal melanin is higher at shorter wavelengths such that if one can take advantage of the longer wavelength HbO_2 absorption bands, less heating of the epidermis will occur, and a greater fraction of the incident energy will be transmitted to the dermis. Given that the extinction coefficients of HbO_2 at the 542- and 577-nm absorption bands are comparable, it is logical to choose the 577-nm wavelength. The wavelengths currently used with the argon laser (488 and 514 nm) are fortuitously located in a region between absorption bands of HbO_2 and within the absorption bands for bilirubin, which is both intravascular and extravascular.

The size of the targeted structures should be commensurate with absorption of a significant fraction of the radiation incident upon it. The ectatic vessels associated with the red lesions of older patients are typically 50–100 μm in diameter (100). At 577 nm, the absorbed fraction of radiation incident on such vessels can be estimated to be 30–50%, based on an effective hemoglobin concentration of 2×10^{-3} M and molar extinction coefficient of 1.52×10^{4} at 577 nm. For vessels of 20–50 μm diameter, typical of younger patients, the estimated absorbed fractions are 16–30%, respectively.

Exposure duration determines the degree of selective heating of tissue immediately surrounding the absorbing chromophores, as compared with the heating of large tissue volumes heated to temperatures sufficient to cause thermal damage. If the exposure duration is long compared with the time required for diffusion of a significant quantity of heat to structures, such as the epidermis, which are some distance away from the target chromophore, then thermal damage will be extensive and nonspecific regardless of how carefully one has chosen a wavelength for heating of the target structure. This is because the absorbed energy will be invested almost uniformly in heating of the tissue during exposure, despite its origin in the target structure. However, if the incident energy is delivered within the time corresponding to retention of heat within the target structure, a maximum, transient temperature differential

and one would not expect to obtain a uniform-thickness layer of the applied material. Furthermore, many sunscreening compounds crystalize on the skin surface after evaporation of the solvent. Both of these mechanisms would act to create a distinctly nonuniform distribution of sunscreen, and result in significantly greater transmission of radiation than would be obtained for a uniform layer. Other possible losses of protection result from either the washing or rubbing off of the sunscreen, or diffusion of the sunscreening compound through the stratum corneum and into interstitial and/or intracellular fluids of the epidermis (92).

Increasing Photobiologic Sensitivity

Prolonged application of water or aqueous media to normal Caucasian skin results in the extraction of UV-absorbing compounds from the skin both in vivo and in vitro. This results in a corresponding increase in transmittance of the stratum corneum as appropriately measured in vitro, and an increase in sensitivity to UVC and UVB radiation, as measured by decreases in MEDs of fair-skinned subjects (93, 94). In contrast, lipophilic substances such as mineral oil neither extract significant amounts of UV-absorbing material from normal skin nor affect the MED to UVB radiation when applied in vivo. The broad absorbance maximum of the cutaneous materials extracted into topically applied water occurs between 265 and 275 nm and correlates both spectrally and quantitatively with the decrease in optical density of human stratum corneum measured in vitro after application and removal of water. The kinetics for extraction of the UV-absorbing compounds into water are such that application times of ½ hr or more are necessary to observe decreases in the MED to UVB radiation from FS40 lamps of 20% or more. The maximum decrease in MED to UVB radiation obtained was a 40–50% decrease after 4 hr of continuous application of water (94). Application of aqueous 5% lactic acid appears to act similarly to water application.

These observations show that extraction of water-soluble, i.e., relatively polar, freely diffusable, UV-absorbing compounds from skin accounts for the increased sensitivity of skin to UVB radiation after soaking the skin for prolonged periods. Chromatographic analysis of the extracted materials indicated that, while most of the material extracted was lipid or protein, a small fraction (about 0.2%) of the material was urocanic acid. Because of its high extinction coefficient (18,800 $mol^{-1} cm^{-1}$ at 277 nm, pH 7.4), however, this small quantity of urocanic acid accounts for approximately 75% of the optical absorbance of the extracted materials. As has been suggested (56, 57), urocanic acid appears to play some role as an "endogenous sunscreen" in human skin. In addition to its epidermal synthesis, urocanic acid is also present in human sweat (56), and is deposited on the skin surface after profuse sweating. Because extensive exposure to sunlight is often associated with

sweating, it is possible that sweating may serve to some extent as a thermall induced photoprotective mechanism.

Normal skin has a single, continuous, though somewhat irregular air tissue interface, in which the refractive index (n_D) changes from that of air, 1.(to that of stratum corneum, 1.55 (20). As discussed above, this causes a regula reflectance of 5–7% across the UV, visible, and near-infrared spectra. Addin a layer of some clear, lipophilic liquid such as mineral oil, which readil spreads over the surface of skin and has a refractive index (n_D = 1.48) betwee that of air and stratum corneum, does little to reduce regular reflectanc occurring at the optical interface with air. However, if there are multipl air–tissue interfaces along the path of incident radiation as it enters th stratum corneum, regular reflectance occurs at each such interface, and th total regular reflectance is considerably greater than for a single air–tissu interface. When mineral oil is applied to the scaly plaques typical of psoriasi vulgaris, it apparently fills air spaces between superficial flakes of corneo cytes. In this case, the regular reflectance of the plaque is decreased afte application of the oil because the oil provides a much better match o refractive index *between* the flakes of corneocytes than does air. Reducing regular reflectance in this manner must necessarily increase the fraction o incident light transmitted into the tissue.

Typical spectral remittance of psoriatic and normal Caucasian skin before and after application of mineral oil is shown in Fig. 6-16 (95). The significant, broad-spectrum decrease in remittance of plaques occurs within seconds after application of oils. Other optically transparent oils produce essentially the same results, but application of water requires up to several minutes to produce a similar effect. The extremely broad spectral character of the decrease in remittance, and the fact that it occurs immediately after application of oils, are entirely consistent with the refractive index-matching mechanism proposed above. Water does not spread readily on the skin surface compared with less polar liquids, which may explain why a longer application time is required for water to cause a similar effect as oils.

The decrease in regular reflectance of psoriasis vulgaris plaques after treatment with oils is typically 10–15%, but varies from as low as 5% decrease to as much as 25% decrease. The expected increase in the fraction of incident radiation transmitted into the psoriatic stratum corneum is essentially the same, 10–15%. However, the increase in that fraction of relatively collimated incident radiation reaching viable keratinocytes is probably considerably greater than 10–15%, perhaps by several times. This may occur because the oil will also drastically reduce off-axis refraction of the incident radiation as it traverses the air–tissue interfaces, resulting in a shorter average pathlength within the stratum corneum, and hence less absorption. Measurements of increased transmittance through psoriatic stratum corneum as a result of application of oils are yet to be accomplished.

of measuring melanin pigmentation or vasodilatation remains to be accomplished. In theory, it should be possible to estimate absolute states of vasodilatation and melanin pigmentation from remittance spectra by calculation of a dermal and epidermal absorption coefficient at wavelengths of strong and weak absorption by blood, respectively. To do this, one must be able to treat dermal scattering (S) as a known parameter, to measure T_r separately from R_D as discussed above, and correct for or eliminate regular reflections occurring at the skin–air interface. This seemingly more complex approach offers the potential advantage of arriving at measurements of cutaneous chromophores that relate directly to their effects upon optical radiation transfer in the skin. Second because the average dermal pathlength involved varies inversely as a function of wavelength, determined by S and K, it is also theoretically feasible to at least monitor changes in vasodilatation as a function of depth within the dermis (21). This may be possible in practice because hemoglobin and oxyhemoglobin have discrete, widely spaced absorption bands over the 400–1000-nm region. Dermal penetration of radiation over this wavelength region varies by more than an order of magnitude (cf. Table 6-2). Therefore, the influence of blood on in vivo remittance at the HbO_2 Soret absorption band near 420 nm corresponds only to blood in superficial vesels (i.e., <100 μm dermal depth). The influence at longer wavelength bands corresponds to blood in vessels at successively greater depths, up to several millimeters in the near-infrared region.

Several studies have shown that in vivo remittance spectra in the 400–510-nm region can be used to noninvasively estimate serum bilirubin levels. Ballowitz and Avery (81) showed that the depression of neonatal skin remittance over the absorption band of bilirubin centered near 460 nm ($\epsilon \simeq 60{,}000$ mol^{-1} cm^{-1}) apparently correlated with serum bilirubin levels in jaundiced infants. Subsequently, Bruce (82) developed an instrument and data analysis for estimation of serum bilirubin levels which gave 95% confidence limits as low as ± 2.2 mg/100 ml for Caucasian neonates not receiving phototherapy. For infants receiving phototherapy, the correlation was poor, presumably due to induction of melanin pigmentation and transients in the distributions of free vs. albumin-bound bilirubin, which have different absorption spectra. The author suggests that maintaining an unexposed test patch during phototherapy might reduce these errors. Data analysis was accomplished by fitting coefficients to a linear summation of remittances at 424, 465, 511, 556, and 629 nm to obtain the best correlation between this function and measured serum bilirubin. Using a similar estimation technique, Hannemann et al. (83) also correlated skin remittance at five wavelengths with bilirubin levels in 56 Caucasian neonates, with ± 2 mg/100 ml 95% confidence limits. These authors, using somewhat different wavelengths (425, 460, 525, 535, and 545 nm) found in addition that a polynomial nonlinear regression analysis gave a slightly better correlation with measured serum bilirubin that did multiple linear regression such as used by Bruce.

Photography is in essence a recording of remittance in which the image is preserved. By placing spectral filters over the camera lens, one can record images of skin remittance of wavelengths of choice across the UV, visible, and near-infrared spectral regions. Judicious choice of wavelength can therefore allow one to enhance the appearance of various pigments or structures. Applications of this technique include UVA photography for visualizing epidermal melanin (76) and near-infrared photography (around 900 nm) for visualization of the deeper cutaneous vessels, even in blacks (84). It has been suggested that near-infrared photographs of pigmented lesions can be used to screen for malignant melanomas, which appear to have less remittance in this region than benign pigmented lesions (85). Whether this effect is due to some difference in the absorption spectrum of melanin produced by malignant melanoma cells or differences in the vascularization or distribution of melanin between benign and malignant lesions is unknown. The recent advent of computerized spectral image-processing systems may some day offer an analogous but sophisticated and diagnostically more powerful tool for visualization of cutaneous pathologies. These devices can enhance and display real-time images based upon computerized analysis of spectral images. To our knowledge, such systems have yet to be applied in any detail for cutaneous analyses.

In Vivo Fluorescence of Skin: Wood's Lamp

In 1908, R. W. Wood (86) placed a UVA-transmitting, visible-absorbing filter over a mercury discharge lamp, and noted that the device caused visible luminescence of many objects, including skin. Dermatologists have since used "Wood's lamps" to diagnose tinea capitis, erythrasma, and some *Pseudomonas* infections, and the appearance of porphyrins in hair, skin, or urine (87). Another application of Wood's lamp is that of visualizing subtle changes in epidermal melanin pigmentation and assessing whether pigmented lesions are due to epidermal vs. dermal deposition of pigment (77). Variations in epidermal pigmentation are much more apparent under Wood's lamp than under visible light. In contrast, lesions due to dermal melanin pigmentation, which often have a blue hue as discussed above, are either less apparent or vanish entirely when viewed under Wood's lamp.

If one sections fresh, lightly pigmented Caucasian skin in vitro and observes the intensity of visible autofluorescence under a Wood's lamp, it is apparent that the dermis fluoresces brightly, while epidermal autofluorescence is comparatively much less. Furthermore, if the entire epidermis including basal layer is separated from the dermis by suction, the separated epidermis alone shows little fluorescence under Wood's lamp, but when placed back

and abdomen postmortem indicate that up to 1% (10^{-2}) of 650–850-nm radiation may reach internal organs, whereas the chest wall transmittance of wavelengths less than 550 nm is less than 10^{-6}.

BIOMEDICAL APPLICATIONS INVOLVING CUTANEOUS OPTICS

In Vivo Remittance Spectroscopy

Absorption bands related to each of the major dermal chromophores (cf. Fig. 6-14) can be distinctly seen as minima in the spectral remittance of Caucasian skin in vivo (13, 70–73). Because melanin lacks discrete absorption bands but absorbs more strongly at shorter wavelengths, its effect is to decrease remittance at shorter wavelengths more so than at longer wavelengths (cf. Fig. 6-11). Accurate measurements of skin remittance at carefully chosen wavelengths can in theory be used to quantify or monitor changes in cutaneous pigments. Possibilities for this general technique therefore include determinations of states of vasodilatation (erythema), oxygen saturation of cutaneous blood, bilirubin levels, and melanin pigmentation. Over the past approximately 50 years, all of these measurements have been attempted with varying degrees of success depending mainly upon each author's choice of wavelengths and ability to interpret the data obtained. Therefore, before examining each measurement scheme specifically, a more general model for the remittance of human skin will be presented.

Over the broad spectral region of approximately 350 nm to approximately 1300 nm, the remittance of skin can be qualitatively and to a large extent quantitatively modeled by considering the thin epidermis to be an optically absorbing element with negligible scattering, overlying the thick dermis, which acts as a diffuse reflector (21). Normally, epidermal transmittance can be assumed to depend only upon melanin content and distribution. Remittance of the dermal element depends upon both scattering, mainly by collagen, and absorption, mainly by the dermal pigments of interest. The dermal remittance component must pass back through the epidermis and eventually through the skin surface to the outside world, to be measured as remittance in vivo.

The justification for neglecting any remittance due to scattering within the epidermis is apparent in Fig. 6-15, which compares the spectral remittance of full-thickness Caucasian skin in vitro with the spectral remittance of the heat-separated epidermis alone placed in optical contact with a black glass background (21). Over the entire 350–1300-nm region, the epidermal remittance is essentially only that due to regular reflectance at the skin surface. The much higher values for full-thickness skin remittance must therefore be due to radiation that has passed through the epidermis, been backscattered within the dermis, and passed through the epidermis once again to be measured.

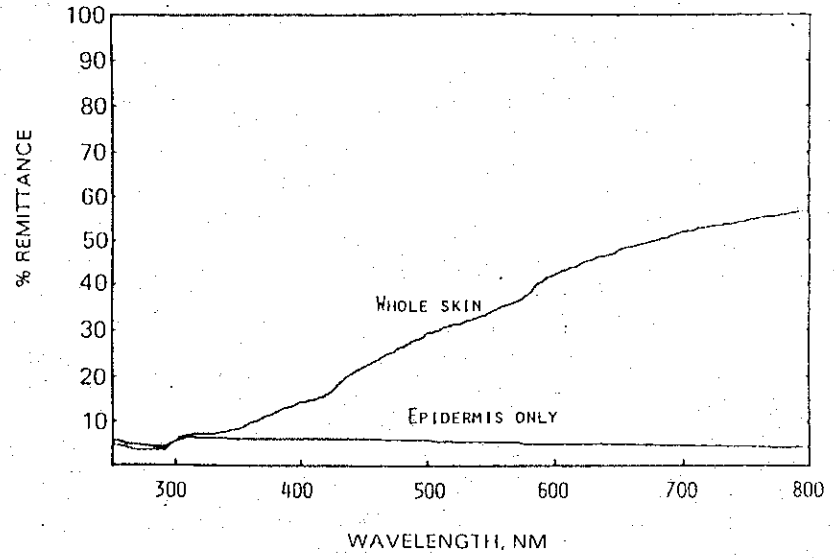

Fig. 6-15. Spectral remittance of full-thickness Caucasian skin (abdominal) in vitro, compared with spectral remittance of the isolated epidermis alone, from the same tissue. The remittance of the epidermis is essentially only that due to regular reflectance. The higher values for full-thickness skin remittance at wavelengths greater than 350 nm are therefore due to radiation that traverses the epidermis, is backscattered from the dermis, and returns through the epidermis once more.

Since this simple model of skin remittance neglects scattering within the epidermis, the effective pathlength of the diffuse radiation returning from the dermis on its second pass through the epidermis is roughly twice that of the epidermis (23, 24). Let us neglect regular reflectance at the skin surface for the moment, and let T_e represent epidermal transmittance for a collimated, normally incident beam, and R_D represent the dermal remittance if the epidermis were not present. The remittance of skin in vivo is then simply $T_e^3 R_D$ (cf. Fig. 6-16). This is derived by inspection as follows: the fraction of the collimated incident flux reaching the dermis is T_e; the dermis then returns a fraction R_D such that the outward-directed diffuse flux at the bottom of the epidermis is $T_e R_D$; finally, this diffuse flux is attenuated by a factor T_e^2 (squared because the effective pathlength is twice as great for the now diffuse radiation) in passing through the epidermis, such that the skin's remittance is $T_e^3 R_D$. To complete this oversimplified model, the effects of regular reflectance occurring at the skin–air interface must be included, both for the incident beam (R_{reg} = 5–7% for a collimated, normally incident beam) and for the diffuse flux encountering the same surface from within the tissue. When one then considers the effects of regular reflection occurring at the skin–air interface, the expression for remittance is complicated by an infinite series of terms representing multiple passes of radiation through the epidermis (21),

dermis must vary inversely with wavelength. Thus, the average dermal pathlength of the scattered light remitted at shorter wavelengths is much less than that of longer wavelengths. Blue light therefore encounters less melanin than red light, and may therefore suffer less absorption. Such scattering is the only means by which a pigment, such as melanin, which absorbs shorter wavelengths more strongly than longer wavelengths, can produce blue colors.

Anderson et al. (21) have presented calculations of spectral scattering (S) and absorption (K) coefficients for human dermis in vitro by application of a modified Kubelka–Munk theory to measurements of transmittance and remittance of thin dermal sections. Measurements were made under conditions appropriate to the assumptions inherent in this model. Measurements of the spectral transmittance and remittance of a typical 200-μm-thick human dermal section, analogous to those of Findlay, are shown in Fig. 6-12. Calculated values for S and K are shown in Fig. 6-13. As expected from Findlay's work, dermal scattering is markedly increased at shorter wavelengths. The absorption coefficient K for bloodless dermis is smaller than S except at the prominent absorption bands of water in the infrared region. Dermal scattering therefore plays a major role in determining the depth to which radiation of various wavelengths penetrates the dermis, and largely accounts for the observations of Hardy et al. (12) and others (31–33, 35) that, in general, longer wavelengths across the UV–visible–near-infrared spectrum penetrate the dermis to a greater extent than do shorter wavelengths.

In vivo, the blood-borne pigments hemoglobin, oxyhemoglobin, beta-carotene, and bilirubin are the major absorbers of visible radiation in the

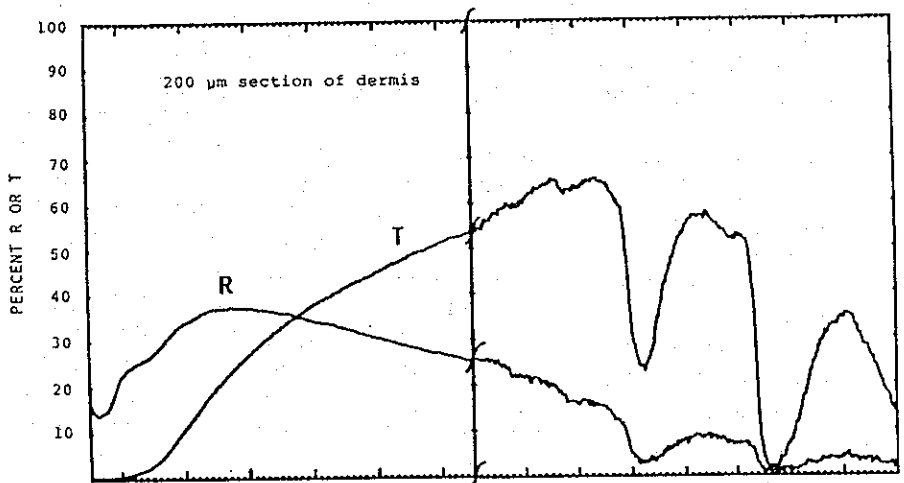

Fig. 6-12. Spectral transmittance and remittance of 200-μm-thickness section of human dermis.

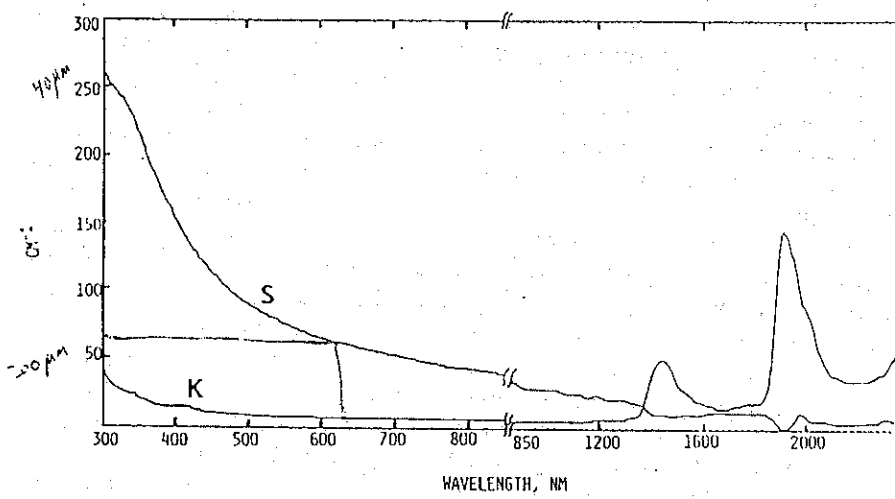

Fig. 6-13. Diffuse scattering (S) and absorption (K) coefficients for human dermis in vitro calculated from measurements of spectral remittance and transmittance of thin dermal sections under conditions appropriate to application of the Kubelka–Munk theory of radiation transfer (20).

dermis. Absorption spectra for these dermal chromophores and for melanin are shown in Fig. 6-14. In addition, typically less than 1% of the total hemoglobin in blood is methemoglobin, which has an absorption band in the red visible region. The effect of these substances on K is not seen in the in vitro dermal spectra presented above, but can be estimated or inferred from in vivo remittance spectra (21, 62). Estimates of the depth at which various wavelengths are attenuated to $1/e$ (37%) of the (diffuse) incident energy density in fair-skinned Caucasians in vivo are given in Table 6-2 (R. R. Anderson, unpublished calculations). The depth was estimated using Eq. (8), assuming known values for S from in vitro studies of dermis to remain constant in vivo, and calculating K in vivo from measurements of R. Vitiligo skin was studied in order to avoid the influence of melanin, such that the $1/e$ penetration depths given in Table 6-2 represent estimated maximum values.

While all of the various forms of hemoglobin are entirely intravascular pigments, bilirubin occurs in both albumin-bound and free forms, and is therefore both intravascular and extravascular. The fraction of bilirubin that is extravascular may be influenced by both vascular permeability and, in hyperbilirubinemia, saturation of albumin binding sites (63). Beta-carotene is highly lipophilic and is sequestered in subcutaneous fat, dermal lipid, and to some extent stratum corneum. Most other mammals retain relatively little beta-carotene and have white subcutaneous fat as opposed to the yellow fat found in humans. These physical distributions of the dermal pigments have great importance from an optical point of view. First, a pigment's physical cross section is important. For example, absorption by hemoglobin and by

protection compared with the induction of melanogenesis. A thorough test of these simple ideas has yet to be demonstrated in vivo.

Contrary to popular beliefs, melanin is not a "neutral density" filter of the skin. Absorption by melanin increases steadily toward shorter wavelengths over the broad spectrum of 250–1200 nm. In the near-infrared beyond about 1100 nm, the absorbance of melanin is essentially negligible. For wavelengths longer than 1100 nm, both transmittance (12) and remittance (47, 48) are unaffected by melanin pigmentation. Figure 6-11 compares the spectral remittance of dark Negroid and fair Caucasian skin. The prevalence of sunburn, abnormal photosensitivity, skin cancers, and cutaneous "aging" decreases with increasing melanin pigmentation. Despite these apparent inverse correlations between skin sensitivity to UV radiation and melanin content, there remain many fundamental questions about how the distribution and quantity of melanin relate to its photoprotective effects. Although melanin may theoretically protect keratinocytes from some UV-induced damage mechanisms by undergoing oxidation–reduction reactions or acting as a free radical quencher, it is apparent from studies of epidermal transmission that absorption of UV radiation primarily accounts for melanin's photoprotective effects. Furthermore, it is well established that, in normal skin, the majority of the melanin present is located in the basal cell layer, and often appears histologically as "caps" of pigment granules overlying the nuclei of many basal cells (49). The majority of basal cells, which are in a resting state, may derive more photoprotection against nuclear DNA damage

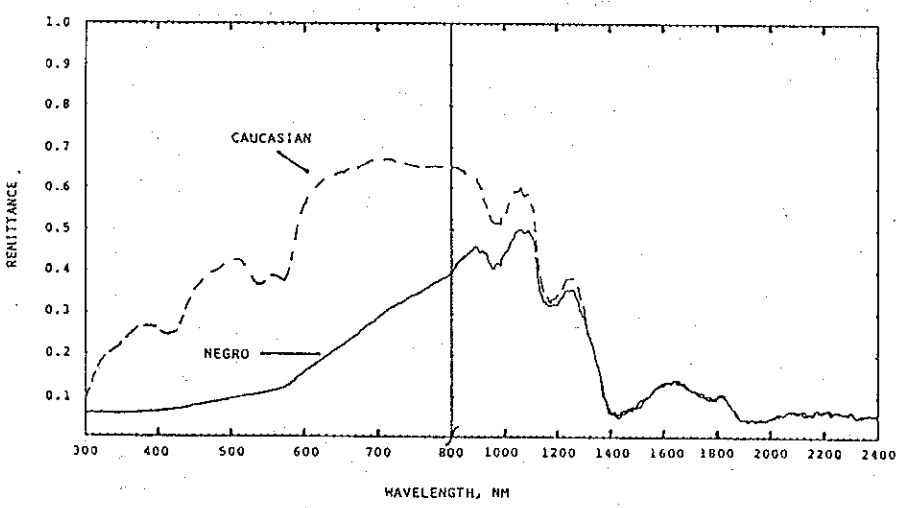

Fig. 6-11. Spectral remittance of dark Negroid and fair Caucasian skin (flexor surface of forearm in each case). The lack of significant absorption by melanin for wavelengths longer than approximately 1100 nm and increased absorption at shorter wavelengths are apparent. Note also that remittance is never less than 5% due to regular reflectance.

by this supranuclear intracellular distribution of melanin than by a random intracellular distribution.

There are no significant racial differences in the numbers of melanocytes per unit area of skin (50) or in the rate of corneocyte loss, although Negroid stratum corneum may have greater physical density (46). Hence, racial differences in melanin content of the skin must be due mainly to differences in the rate at which individual melanocytes produce and transfer melanin to keratinocytes, and to some extent racial differences in melanosome distribution.

Melanin is a remarkably 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, and also appear to suffer greater degradation within keratinocytes (51). The above considerations account for the observation of free "melanin dust" in the Caucasian stratum corneum, whereas Negroid stratum corneum contains numerous intact melanosomes in addition to "melanin dust" (14, 52). The optical effects associated with dispersed "melanin dust" vs. intact melanosomes have not been quantitated, but it is likely that dispersal of melanin pigment in the Caucasian stratum corneum affords somewhat greater protection than would the same quantity of melanin sequestered in intact melanosomes. This is because the pigment presents a greater physical cross section to optical radiation. Interestingly, Caucasian skin sites exposed to UVA after oral administration of photosensitizing psoralens (52) are induced to produce single-granule melanosomes similar to those of Negroid skin.

Given that, across the range of human pigmentation, there are gross differences in the dispersion and concentration of melanin and the stability of melanosomes, and that melanin is preferentially concentrated in the basal cell layer, there exist grossly different distributions of internal optical dosimetry between heavily pigmented skin and lightly pigmented skin. Furthermore, such differences, if quantitatively correlated with variations in thresholds for photobiologic responses, may give clues to the location of tissue layers involved in initiating photobiologic responses. In an attempt to correlate the photoprotection offered by melanin with its quantity and distribution, Kaidbey et al. (53) measured the UVB and UVA spectral transmittance of Caucasian and Negroid stratum corneum and epidermis samples. In addition to spectrophotometric measurements of transmittance, the minimal delayed erythema doses (MEDs) of broadband UVB from fluorescent sunlamps and UVA from a filtered xenon source were determined with and without the skin samples placed over test sites on fair Caucasian subjects. The transmittance of erythemogenic radiation through each skin sample was taken to be the ratio of MED without divided by MED with the sample placed over the subject's skin. Both the UVB and UVA transmittance values measured in these two ways were in general agreement with the UVB and UVA transmittances measured

determining the transmission of optical radiation through the stratum corneum and epidermis. The high absorbance of epidermis and stratum corneum for wavelengths less than 240 nm is largely due to peptide bonds.

Two technical problems have plagued all previously reported epidermal or corneal transmission spectra, however. First, the tissue is autofluorescent, primarily because of aromatic amino acids (38) and perhaps melanin as well (39). A broad excitation band centered near 280 nm is associated with an emission band between 330 and 360 nm, consistent with tryptophan or tyrosine fluorescence (R. R. Anderson, unpublished observations). This emission band is of sufficient intensity to cause suspicion of epidermal transmission spectra taken with integrating spheres equipped with broadband (UV–visible-sensitive) photomultiplier detectors, as in essentially all standard commercially available systems. The portion of absorbed 260–290-nm radiation which is emitted as fluorescence at 330–360 nm is falsely recorded by the broadband detector as transmittance at 260–290 nm. The comparatively high epidermal transmittance at the 330–360-nm fluorescence emission band can cause order-of-magnitude errors in the measurement of 260–290-nm epidermal transmittance, especially if the samples are heavily pigmented. Second, if the epidermal sheet is simply suspended in air or placed against a quartz slide at the entrance port of an integrating sphere, as has been the case in previous studies of epidermal transmittance, some total internal reflection of forward-scattered off-axis rays can occur, which are then lost for measurement purposes.

The autofluorescence error has been overcome by using a "solar-blind" detector, which is insensitive to wavelengths longer than 320 nm. The problem of total internal reflection has been overcome by building a chamber in which epidermal samples are floated over a hemispherical-shaped quartz chamber containing normal saline. This apparatus is shown diagrammatically in Fig. 6-8. Representative fair-skinned Caucasian epidermal and stratum corneum transmission spectra taken with this system, and expressed in absorbance or optical density (OD = $-\log T$) units, are presented in Fig. 6-9 and compared with conventional spectra not corrected for autofluorescence.

In Caucasians, roughly half of incident 320–400-nm (UVA) radiation reaches the dermis (1). Furthermore, although only a small fraction of incident radiation of wavelengths less than 300 nm reaches the dermis, it cannot be assumed that these wavelengths exert no direct dermal effects. Delayed erythema induced by 254-nm radiation (Caucasian epidermal transmittance 1%) may involve direct damage to dermal vessels (40). A transient decrease in circulating T lymphocytes is caused by total-body 290–320-nm (UVB) exposure (41), or 320–400-nm (UVA) exposure after ingestion of psoralens (42) at incident exposure doses that are consistent with direct damage to these cells, based upon Caucasian epidermal transmittance and in vitro dose–response studies of lethality or induction of DNA repair in T lymphocytes irradiated in serum-free media (43) (cf. also Chapter 10).

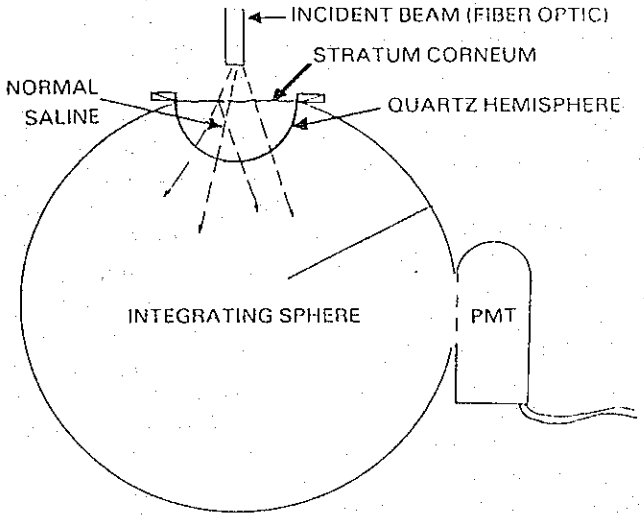

Fig. 6-8. Apparatus for appropriate measurement of spectral transmission through stratum corneum or epidermis. Because of autofluorescence of the tissue, the photomultiplier detector (PMT) should be of a "solar blind" type (Cs–Te photocathode material) for measurements at wavelengths less than 320 nm. The hemispherical shape of the chamber holding the skin and the presence of saline beneath the sample are necessary to avoid errors due to regular reflectance at the bottom surface of the sample, rendering the measurement, and the sample's environment, analogous to the in vivo situation.

For reference, absorption spectra of major epidermal pigments are given in Fig. 6-10. It can be seen upon careful inspection of these data that epidermal or corneal transmittance spectra are compositely determined by absorption by these (and certainly other) substances. It is largely variations in the concentrations, distributions, or amounts of these chromophores that determine individual and anatomic variations in epidermal spectral transmission. One would expect the protein-bound and nucleic acid-bound chromophores to be of rather constant concentration and distribution in normal skin since these chromophores are necessarily inherent to the cellular matrix of the tissue. As such, the magnitude of the 275-nm epidermal absorption maximum varies almost linearly with variations in epidermal or corneal thickness (R. R. Anderson, unpublished observations). For both melanin and urocanic acid, however, their concentrations and distributions are variable, and unlike protein or nucleic acid, UV optical absorption may be their major functional role in human skin.

Melanogenesis is well known to be induced or enhanced by UV radiation exposure with or without photosensitizers, whereas there are conflicting reports on the possible photoinduction of urocanic acid synthesis in the epidermis (44, 45). Thomson (10) showed clearly that racial variations in epidermal melanin content account for a much greater effect on the optical

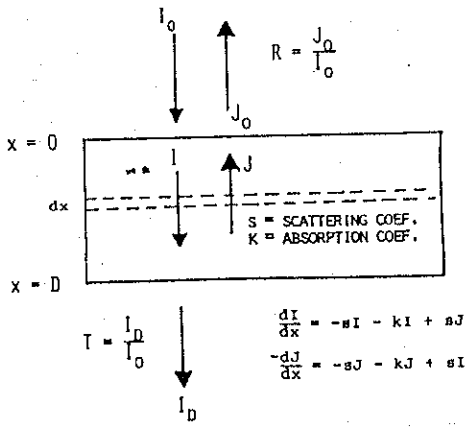

Fig. 6-6. The Kubelka–Munk model for radiation transfer in a scattering, absorbing medium. I and J are defined as diffuse fluxes, and S and K are backscattering and absorption coefficients for diffuse radiation, respectively.

thickness; (2) the incident radiation is diffuse; and (3) differences in refractive index between the sample and external medium, causing regular reflectances, are neglected. Radiation within the sample is broken simply into two opposing diffuse fluxes, I and J in Fig. 6-6. The sample's backscattering (S) and absorption (K) coefficients for diffuse radiation are defined by two differential equations as the fraction of diffuse radiation either backscattered or absorbed per unit differential pathlength of the sample. These differential equations are

$$dI = (-KI - SI + SJ)\,dx \tag{1}$$

$$-dJ = (-KJ - SJ + SI)\,dx \tag{2}$$

which simply state that the change dI in flux I over some layer of thickness dx is equal to that fraction of I removed by absorption and backscattering plus some fraction contributed to I by backscattering from J. The second differential equation is an analogous statement for flux J. The minus sign in Eq. (2) is necessary because J is defined as positive in the minus x direction.

The differential equations (1) and (2) are solved to give a general solution of the form

$$I = A(1 - \beta)e^{\alpha x} + B(1 + \beta)e^{-\alpha x} \tag{3}$$

$$J = A(1 + \beta)e^{\alpha x} + B(1 - \beta)e^{-\alpha x} \tag{4}$$

where $\alpha = [K(K + 2S)]^{1/2}$ and $\beta = [K/(K + 2S)]^{1/2}$.

The boundary conditions are that $T = I_d/I_0$, $R = J_0/I_0$, $I_0 = I_0$, and $J_d = 0$, where R and T are remittance and transmittance of the sample, respectively, and the subscripts represent values of x. Substitution of these

boundary conditions gives a particular solution, which can then be rearranged to express S and K (our two unknowns) in terms of R and T (our measurable quantities). Written in the forms derived by Kubelka and Munk, these are

$$\frac{K}{S} = \frac{1 + R^2 - T^2}{2R} - 1 \tag{5}$$

$$S = \frac{1}{d}\left(\frac{K}{S(K/S + 2)}\right)^{-1/2} \coth^{-1}\frac{1 - R(K/S + 1)}{R[K/S(K/S + 2)]^{1/2}} \tag{6}$$

Because a minimum of two different measurements is always necessary to determine two unknowns, measurement of both remittance and transmittance (or, alternatively, remittance with two different reflective "backgrounds" of different reflectance behind the sample) is required. In order to use this model practically, one must also account for regular reflection occurring at both sample boundaries and adhere to the use of diffuse incident radiation. This has been accomplished for dermal samples in vitro (21), and S and K for human dermis devoid of blood have been calculated. In vivo, blood exerts major influence upon K, which thus far has been only crudely estimated from in vivo remittance spectra of depigmented skin. Once S and K are known, the two fluxes I and J can be reconstructed via Eq. (3) and (4). The total density of optical radiation at a given depth is the algebraic sum of I and J at that depth; it is this sum that determines optical dosimetry within the layer. For an infinitely thick sample (in practice, a sample for which T approaches zero), $A = 0$ and $B = 1/(1 + \beta)$ in Eq. (3) and (4). The sum of I and J is therefore

$$I + J = \frac{2e^{-\alpha x}}{1 + \beta} \tag{7}$$

and the depth at which the radiation density is attenuated to $1/e$, or 37%, of its incident value is

$$d(37\%) = \frac{1}{\alpha}\left(1 + \ln\frac{2}{1 + \beta}\right) \tag{8}$$

Near the front surface of a scattering sample, the sum of I and J can easily exceed I_0, the incident density of optical radiation. In the extreme case, I plus J just inside the surface of a sample with 100% remittance is twice I_0, since $I_0 = I_0$ at the surface. Thus the density of optical radiation in the superficial layers of a scattering sample can easily exceed that of the incident beam. As shall be seen this is often the case for fair Caucasian skin, especially over the visible and near-infrared regions.

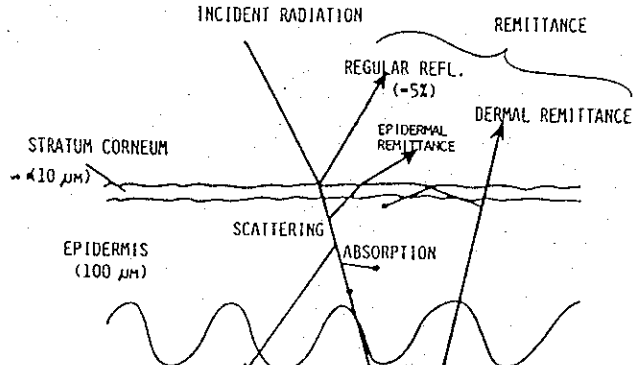

Fig. 6-3. Schematic representation of optical pathways in human skin.

incidence is varied. The main effects of the nonplanar surface topology of the stratum corneum are: (1) that the regular reflectance from skin is not specular, i.e., skin reflectance does not maintain an image, as does the reflectance of a mirror or a window; and (2) that collimated incident radiation, upon passing through this surface and into the skin, is refracted and therefore made somewhat more diffuse by this rough surface. These effects are conceptually similar to those that make a piece of ground glass translucent, compared with the transparency of polished glass.

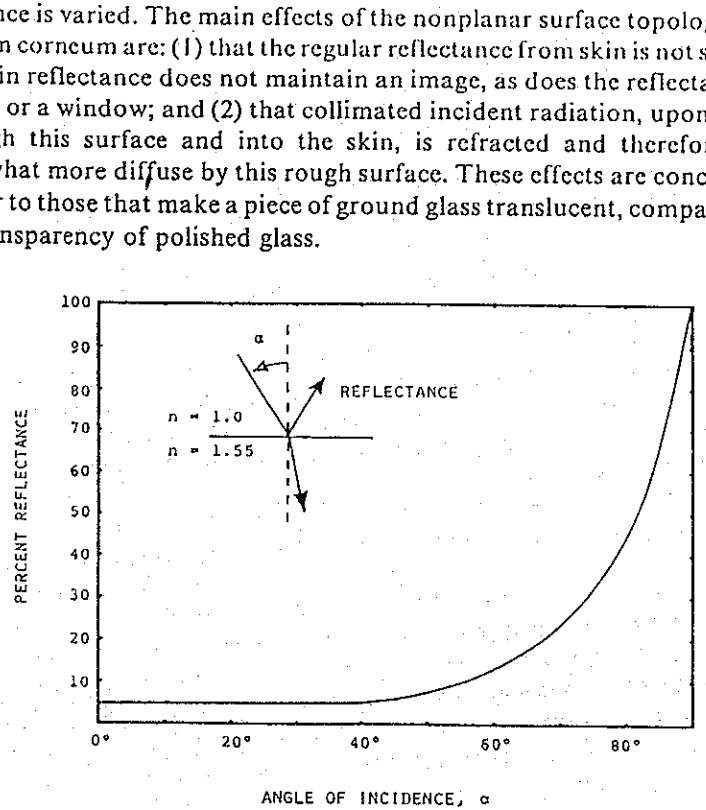

Fig. 6-4. Regular reflectance at a planar interface of a material with refractive index 1.55.

Referring again to Fig. 6-3, and denoting the 4–7% regular reflectance as R_{reg}, we have that the fraction of normally incident radiation entering the skin is simply $1 - R_{reg}$, or 93–96%. Within any of the layers of skin, this radiation may be *absorbed* or *scattered*. These two processes taken together essentially determine the penetration of radiation into the skin. The remittance of the skin is also a function of both scattering and absorption within the various tissue layers to which the radiation penetrates.

Most of the absorbed radiation is rapidly converted by nonradiative deexcitation to heat, which is then dissipated. All biologic effects of optical radiation result directly or indirectly from either photochemical reactions or radiant heating, or both. Heating is initially localized near the pigments that absorb the radiation, but thermal diffusion rapidly spreads the heating over large volumes of tissue. A small fraction of the absorbed radiation, particularly in the UV region, leads to fluorescence, phosphorescence, and the photochemistry that initiates photobiologic responses. All of these three "nonthermal" means of dissipating the electronic excitation produced by photons are of interest in photobiology, but from a functional point of view, photochemistry is most important. The wavelengths producing photobiologic responses (i.e., the action spectrum) are theoretically those that are absorbed by specific molecules or chromophores that initiate the photochemical events leading to the observed biologic responses. An action spectrum may therefore correlate with the absorption spectra of chromophores involved (22), but account must be taken of the optical properties of the tissue itself. Knowing the quantitative transfer of incident radiation to a biologically significant layer or volume, one may correct the biologic action spectrum for tissue optics. Unfortunately, the location of chromophores initiating important responses is often a mystery.

Optics of Turbid Media

Photons (optical quanta) have a rest mass of zero. When a photon is absorbed, its energy is invested in the chromophore molecule, and the photon ceases to exist. Most scattering, on the other hand, is an elastic interaction between optical radiation and matter in which only the direction of photon propagation is altered. Some types of scattering are inelastic and result in a change of wavelength as well; these are of minor consequence in the present discussion. Scattering results from inhomogeneities in a medium's refractive index, corresponding to physical inhomogeneities. The spatial distribution and intensity of scattered light depend upon the size and shape of the inhomogeneities relative to the wavelength, and upon the difference in refractive index between the medium and the inhomogeneities. For molecules or small particles with dimensions less than roughly one-tenth of the wavelength, scattering is generally weak, nearly isotropic (equally distributed

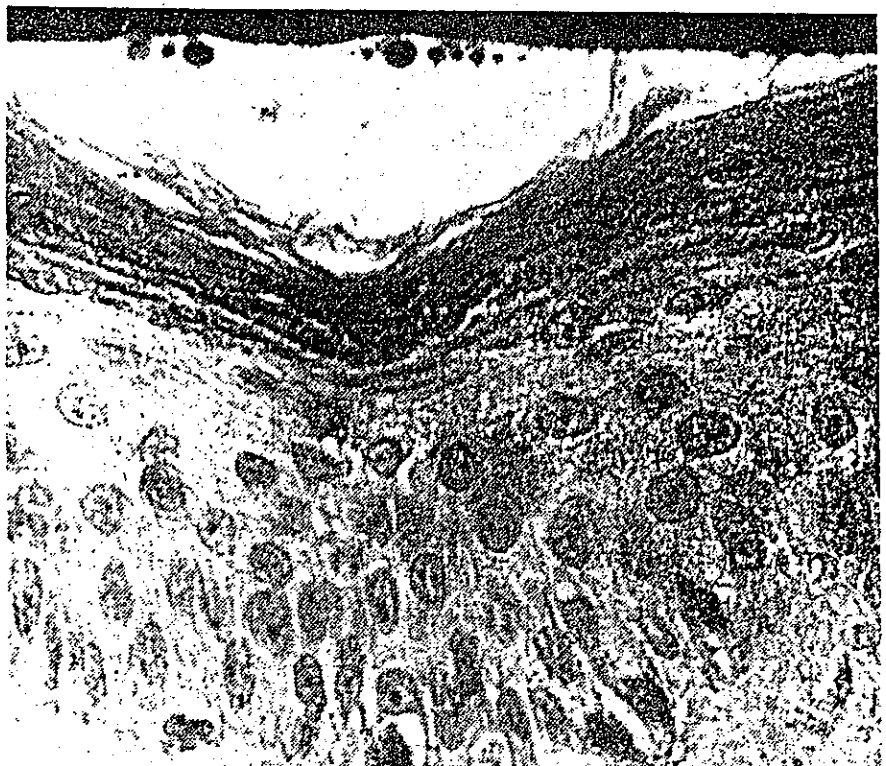

Fig. 6-1. The epidermis.

granulosum. Finally, these cells lose their nuclei, dehydrate, and flatten out into dead, polygonal cells with a surface area about 25 times that of the basal cells. The close packing and cementing of these flat, dead cells laden with keratin form the tough stratum corneum, and the cells are called corneocytes. In normal skin, it may take up to 14 days for a daughter cell of the basal layer to reach the stratum corneum and another 1–2 weeks before it is sloughed off from the skin surface.

Epidermal Melanin Pigmentation

The earliest scientific studies in pigmentation are attributed to Marcel Malpighi (8) and Johann Pechlin (9), who discovered in the 17th century that most of the blackness of Ethiopian cadaver skin was localized in a layer (the basal cell layer of the epidermis) between the white corium (dermis) and an outer "scarf" (the upper epidermis and stratum corneum). It is now known

that this pigment is melanin, an amorphous complex polymer of tyrosin oxidation products and proteins. Specialized cells called melanocytes, derive from neural crest tissue, reside in the basal cell layer of the epidermis an produce melanin-containing granules called melanosomes, which are trans ferred through dendritic processes to the keratinocytes of the epidermis. A the keratinocytes migrate outward, some melanin is carried toward th surface of the skin, eventually appearing in the stratum corneum. Despite th continuous outward migration of melanin, its greatest concentration, as note by Malpighi, is in the basal cell layer. This is primarily due to the presence o heavily pigmented melanocytes and resting basal cells in this germinativ layer.

Melanin strongly absorbs shorter wavelength visible light and UV radiation because of extensively conjugated double bonds within the polymer The protection against sunburn afforded by melanization of the skin has lon been recognized and is experienced seasonally by most people. Thompso (10) and others (11–14) have shown, in excised human epidermis, that melani pigmentation is a greater factor in determining the transmission of UV radiation through the epidermis than is variation in epidermal thickness Protection by melanin and melanin's effects on skin color must be related no only to the quantity of melanin, but to where it is and how it is dispersed. I addition to inducing delayed melanogenesis, epidermal cell kinetics can b markedly altered by UV radiation (15, 16). Given that the quantity an distribution of melanin within the epidermis is dependent upon the dynami equilibrium among its rate of production, its rate of transfer to keratinocytes and its rate of loss as determined by keratinocyte and corneocyte cell kinetics any perturbance of this dynamic equilibrium results in alteration of both th distribution and total amount of melanin. Hence, melanin's effects upo optics of the skin and sunburn protection are determined by a complex o factors, and vary over time.

Precise dose–response, action spectrum, or photoprotective-effect studie for single exposures or for multiple exposures leading to steady-stat equilibria of this interesting photoinducible, photoprotective system ar lacking. The action spectrum for induction of melanogenesis grossly re sembles that for induction of delayed erythema, but at longer wavelengths i the near-UV or visible spectral regions, melanogenesis can be induced b suberythemogenic exposure doses (17, 18). Tanning induced by UVA (320 400 nm) radiation may be less protective against UVB (290–320 nm) radiatio than UVB-induced tanning (19), but if true, the mechanism for this differenc is unknown.

Dermis

The dermis is an optically turbid, semisolid mixture of fibers, cells, an vessels embedded in a viscous gel of water and mucopolysaccharides. Ther