ful for study by three-dimensional reconstruction. They have sufficient order for tilting studies and have relatively strong diffraction to resolutions of 35 to 40 Å. They are composed of *E. coli* components and hence the immunological and biochemical approaches developed for them can be applied to the crystals. For example, specific regions of ribosomes may be labeled by Fab antibody tags against proteins and RNA [for a review, see (*9*)] to determine their positions directly by diffraction techniques. With a three-dimensional map at 50-Å resolution, our preliminary experiments suggest that it is possible to investigate the shapes and mutual orientations of such features as the L7/L12 stalk (*10*), the central protuberance [the likely site of peptidyl transfer (*4*)] and the L1 ridge (*11*). Moreover, the *E. coli* ribosome offers optimal opportunities for correlating results from structural studies with other information. From the degree of order found in these electron micrographs, it also seems possible that single crystals suitable for x-ray diffraction might be obtained.

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References and Notes

1. P. N. T. Unwin, *Nature (London)* **269**, 118 (1977).
2. J. A. Lake and H. S. Slayter, *J. Mol. Biol.* **66**, 271 (1972).
3. M. W. Clark, M. Hammons, J. A. Langer, J. A. Lake, *ibid.* **135**, 507 (1979).
4. J. A. Lake, in *Ribosomes: Structure, Function, and Genetics*, G. Chambliss *et al.*, Eds. (University Park Press, Baltimore, 1979), pp. 207–236.
5. A. E. Yonath, J. Mussig, B. Tesche, S. Lorenz, V. A. Erdmann, H. G. Wittmann, *Biochem. Int.* **1**, 428 (1980).
6. D. J. DeRosier and A. Klug, *Nature (London)* **217**, 130 (1968).
7. The nomenclature of the 17 two-sided plane groups is that of S. P. Fuller, R. A. Capoldi, and R. A. Henderson [*J. Mol. Biol.* **134**, 305 (1979)].
8. R. F. Gesteland, *ibid.* **18**, 356 (1966).
9. D. Winkelmann and L. Kahan, in *Ribosomes: Structure, Function, and Genetics*, G. Chambliss *et al.*, Eds. (University Park Press, Baltimore, 1979), pp. 255–265.
10. W. A. Strycharz, M. Nomura, J. A. Lake, *J. Mol. Biol.* **126**, 123 (1978).
11. J. A. Lake and W. A. Strycharz, *ibid.* **153**, 979 (1981).
12. We thank D. Williams and J. Beyer for electron microscopy and photography. Supported by grant PCM 76-14718 from the National Science Foundation and grant GM-24-34 from the National Institute of General Medical Sciences (to J.A.L.), and by PHS national research award 07104 (to M.W.C.). We thank J. Washizaki for photography and D. Eisenberg and D. L. D. Caspar for advice.

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Spectral Character of Sunlight Modulates Photosynthesis of Previtamin D_3 and Its Photoisomers in Human Skin

Abstract. *The photosynthesis of previtamin D_3 from 7-dehydrocholesterol in human skin was determined after exposure to narrow-band radiation or simulated solar radiation. The optimum wavelengths for the production of previtamin D_3 were determined to be between 295 and 300 nanometers. When human skin was exposed to 295-nanometer radiation, up to 65 percent of the original 7-dehydrocholesterol content was converted to previtamin D_3. In comparison, when adjacent skin was exposed to simulated solar radiation, the maximum formation of previtamin D_3 was about 20 percent. Major differences in the formation of lumisterol$_3$ and tachysterol$_3$ from previtamin D_3 were also observed. It is concluded that the spectral character of natural sunlight has a profound effect on the photochemistry of 7-dehydrocholesterol in human skin.*

Man, evolving in an environment bathed in sunlight, developed a variety of physiological responses to solar radiation. One of the best characterized sunlight-mediated cutaneous events in man is the photosynthesis of vitamin D_3. Exposure to sunlight causes the photochemical transformation of 7-dehydrocholesterol (7-DHC) to previtamin D_3 (preD_3) in human skin (*1*). The preD_3 isomerizes by heat to vitamin D_3 or by ultraviolet (UV) radiation to lumisterol$_3$ and tachysterol$_3$ (Fig. 1A). The isomerizations of preD_3, lumisterol$_3$, and tachysterol$_3$ are photoreversible reactions and therefore determine in part the yield of preD_3 and, ultimately, of vitamin D_3 that is produced in the skin. Equipped with the capability of carefully monitoring this well-defined photochemical event in human skin, we investigated the effect of monochromatic radiation on this photochemical reaction and compared it with that resulting from exposure to simulated sunlight. We observed that there were major differences in both the conversion of 7-DHC to preD_3 and the photoisomerization of preD_3 to lumisterol$_3$ and tachysterol$_3$ in human epidermis exposed to narrow-band (295-nm) radiation compared with epidermis exposed to simulated or natural solar radiation. We report that the spectral character of natural sunlight is an important factor that modulates the photosynthesis of preD_3, lumisterol$_3$, and tachysterol$_3$ in human skin.

Surgically obtained type III human skin was separated by heat (*1, 2*) and then exposed at room temperature to narrow-band radiation, obtained from a 5-kW xenon arc lamp and a monochromator system (Jobin-Yvon HL300), with a half-band width of either 5 or 3 nm. Immediately after irradiation, epidermal lipids were extracted and chromatographed to determine the amount of 7-DHC and its photoproducts (*1–3*). Figure 1B illustrates an action spectrum thus obtained for the production of preD_3 from 7-DHC in human epidermis. The optimum wavelengths for the production of preD_3 are between 295 and 300 nm, with an apparent maximum near 297 nm, results similar to those obtained in the rat and in organic solvents (*4–7*).

Having established that narrow-band, 295- to 300-nm radiation optimally produces preD_3 in human skin, we exposed adjacent, paired samples of human skin to increasing doses of either monochromatic radiation (295 ± 5 nm) or simulated solar UV radiation comparable to that striking the earth at 0° latitude in June at noon (*2*). With exposure to 295-nm radiation, the maximum possible conversion of 7-DHC to preD_3 in human epidermis was approximately 60 ± 5 percent of the original concentration of 7-DHC (Fig. 2A). At this time, a quasi-photostationary state was established with tachysterol$_3$, lumisterol$_3$, and 7-DHC representing 25 to 30, 5 to 10, and 2 to 5 percent, respectively (Fig. 2B). In comparison, when the adjacent skin samples were exposed to an equivalent of 15 to 30 minutes of simulated equatorial solar radiation, the maximum preD_3 produced was only 15 to 20 percent of the original 7-DHC levels (Fig. 2A), and a quasi-photostationary state was established with tachysterol$_3$, lumisterol$_3$, and 7-DHC representing 3 to 6, 50 to 60, and 10 to 20 percent, respectively (Fig. 2). Thus, in comparison with narrow-band 295-nm radiation, exposure to simulated equatorial solar UV radiation significantly diminished the maximum formation of preD_3 in the epidermis and enhanced its conversion to lumisterol$_3$. To determine whether human epidermis itself was responsible for the major differences in photoisomer yield between these sources, crystalline 7-DHC was dissolved in tetrahydrofuran at various concentrations (1 n*M* to 1 m*M*) and exposed to radiation of 295 ± 5 nm or to simulated solar radiation. The difference in the

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shape of the curves (Fig. 2A) for the percent conversion to $preD_3$ from 7-DHC in human epidermis or from 7-DHC in an organic solvent after exposure to narrow-band (295 nm) radiation can be explained by the attenuation of this wavelength by the stratum corneum (*8*). This difference is not seen in the curves after exposure to simulated sunlight (Fig. 2A) because of the presence of high-intensity, more transmissible, longer wavelengths (310 to 340 nm) that are present in the solar spectrum (Fig. 1B).

To be certain that the solar simulator was not in some way inducing this striking difference, we also compared epidermis exposed to natural noontime sunlight on a cloudless June day in Boston with samples exposed to simulated Boston (42° latitude) noontime sunlight (*2*). This comparison showed that the quasi-photostationary states achieved after exposure to natural radiation and to artificial sources of radiation were similar.

These striking differences in photoisomer yields are best explained by the relative overlaps of the radiation between 290 and 340 nm in the solar spectrum with the various absorption spectra of 7-DHC, $preD_3$, $lumisterol_3$, and $tachysterol_3$ within this region. The forward reaction rate for any of the photoisomers (Fig. 1A) is a product of the

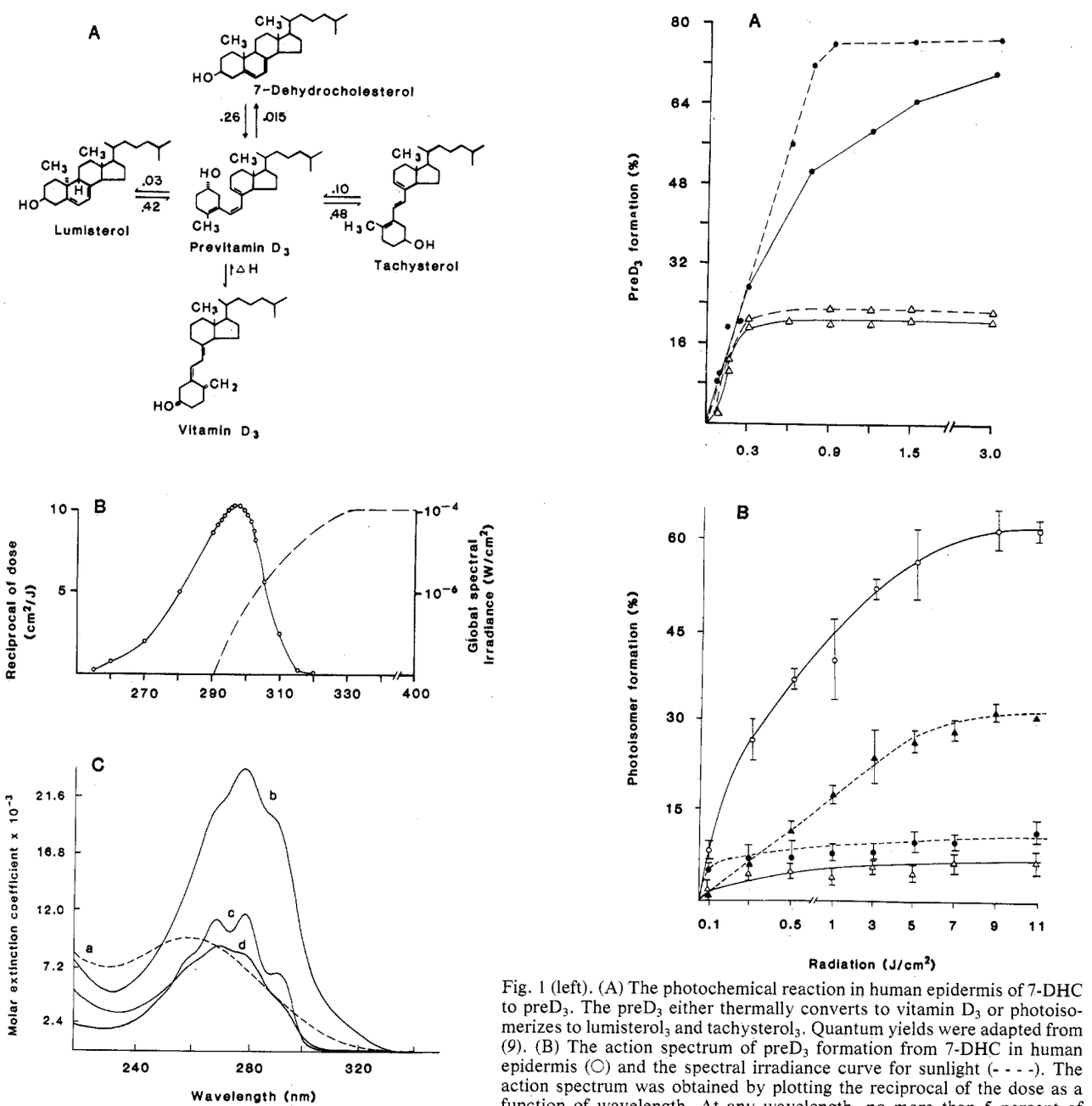

Fig. 1 (left). (A) The photochemical reaction in human epidermis of 7-DHC to $preD_3$. The $preD_3$ either thermally converts to vitamin D_3 or photoisomerizes to $lumisterol_3$ and $tachysterol_3$. Quantum yields were adapted from (*9*). (B) The action spectrum of $preD_3$ formation from 7-DHC in human epidermis (○) and the spectral irradiance curve for sunlight (- - - -). The action spectrum was obtained by plotting the reciprocal of the dose as a function of wavelength. At any wavelength, no more than 5 percent of product was made. The overlay of the curve of the action spectrum with that of the solar spectrum [adapted from (*11*)] demonstrates the small portion of the solar UV spectrum that is involved with the production of $preD_3$ from 7-DHC. (C) Ultraviolet absorption spectrums of (a) $preD_3$, (b) $tachysterol_3$, (c) 7-DHC, and (d) $lumisterol_3$ isolated from human epidermis. Fig. 2 (right). (A) Percent formation of $preD_3$ from 7-DHC in human epidermis (——) or from crystalline 7-DHC (10 μg/ml) dissolved in tetrahydrofuran (- - - -) after exposure to (●) a range of doses of narrow-band radiation at 295 ± 5 nm (*1*) or to (△) simulated solar radiation (*2*). (B) Percent formation of lumisterol (○ and ●) and tachysterol (△ and ▲) in human epidermis after exposure to a range of doses of narrow-band radiation at 295 ± 5 nm (- - - -) or to simulated solar radiation (——) (*2*). The amount of UV is measured by a 295-nm radiometer for the narrow-band source, and the amount of 290- to 302-nm radiation is measured by a radiometer for the simulated solar radiation source.

quantum yield (*9*) for the reaction and the number of photons available to and absorbed by the starting isomer. The number of photons absorbed is, in turn, determined by the overlap of the source's spectral irradiance with the absorption cross section of the starting isomer. Hence, in this reaction, those isomers showing good absorption in spectral regions of high intensity will be preferentially converted to other isomers. Solar spectral irradiance (Fig. 1B) shows an increase of about 3.5 orders of magnitude, from 290 to 320 nm. The UV absorption spectra for 7-DHC, $preD_3$, $lumisterol_3$, and tachysterol (Fig. 1C) demonstrate that 7-DHC and $lumisterol_3$ (in both protic and aprotic solvents) show negligible absorption above 315 nm, whereas both $preD_3$ and $tachysterol_3$ absorb radiation to at least 325 and 335 nm, respectively. Thus, the extinction coefficients for $preD_3$ and $tachysterol_3$ at 320 nm (for example) are relatively high (480 and 1700, respectively) compared with 0.1 or less for lumisterol and 7-DHC. The fact that the solar irradiance at 320 nm is 3.5 orders of magnitude greater than at 290 nm makes these absorption characteristics of $preD_3$ and $tachysterol_3$ significant. Even though the quantum yield for $tachysterol_3$ to $preD_3$ is low (Fig. 1), tachysterol, which has the highest extinction coefficient above 315 nm, is the most photoreactive isomer when exposed to sunlight, and the reaction is therefore driven from $tachysterol_3$ to $preD_3$ to $lumisterol_3$, which accumulates because it is the least photoreactive isomer. To test this hypothesis, we exposed tachysterol and $preD_3$ dissolved in an organic solvent to radiation between 315 and 340 nm and observed an accumulation of $lumisterol_3$.

During the past century, scientists have begun to appreciate several biologic effects of sunlight on the human body (*10*). Stratospheric ozone, a major component in the atmospheric path of sunlight, determines the 290-nm short-wavelength cutoff that is characteristic for the terrestrial solar spectrum (*11*) (Fig. 1B). In addition, the spectral characteristics of natural sunlight that penetrates to the earth's surface vary with altitude, latitude, time of the day, and season of the year. Little is known about whether the spectral properties of sunlight promote unique biologic actions in humans. Our observation that the spectral power distribution of sunlight has a dramatic effect on the cutaneous photosynthesis of $preD_3$ and its photoisomers, however, suggests that the spectral character of the radiation in the surrounding natural and artificial environments may be important for regulating subtle radiation-induced physiological and biochemical responses in humans. Our observations may also be helpful in the design of radiation sources that could enhance the production in vivo of $preD_3$ in human skin and the commercial production in vitro of previtamin D and previtamin D metabolites.

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References and Notes

1. M. F. Holick *et al.*, *Science* **210**, 203 (1980).
2. M. F. Holick, J. A. MacLaughlin, S. H. Doppelt, *ibid.* **211**, 590 (1980).
3. M. F. Holick, N. M. Richtand, S. C. McNeill, S. A. Holick, J. E. Frommer, J. W. Henley, J. T. Potts, Jr., *Biochemistry* **18**, 1003 (1979).
4. A. Knudson and F. Benford, *J. Biol. Chem.* **124**, 287 (1938).
5. J. W. M. Bunker and R. S. Harris, *N. Engl. J. Med.* **216**, 165 (1937).
6. K. Takada, T. Okano, Y. Tamura, S. Matsui, T. Kobayashi, *J. Nutr. Sci. Vitaminol.* **25**, 385 (1979).
7. T. Kobayashi and M. Yasumura, *ibid.* **19**, 123 (1973).
8. J. A. Parrish, R. R. Anderson, F. Urbach, D. Pitts, in *UVA: Biological Effects of Ultraviolet Radiation* (Plenum, New York, 1978), pp. 59–81.
9. E. Havinga, *Experientia* **29**, 1181 (1973).
10. R. J. Wurtman, *Sci. Am.* **233**, 69 (July 1975).
11. W. E. Green, T. Sawuda, E. P. Shettle, *Photochem. Photobiol.* **19**, 251 (1974).
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Decreased Nocturnal Plasma Melatonin Peak in Patients with Estrogen Receptor Positive Breast Cancer

Abstract. *Plasma melatonin concentrations were determined over a period of 24 hours in 20 women with clinical stage I or II breast cancer. In ten of the patients, whose tumors were estrogen receptor positive, the nocturnal increase in plasma melatonin was much lower than that observed in eight control subjects. Women with the lowest peak concentration of melatonin had tumors with the highest concentrations of estrogen receptors. A significant correlation was found between the peak plasma melatonin concentration and the tumor estrogen receptor concentration in 19 of the patients. These data suggest that low nocturnal melatonin concentrations may indicate the presence of estrogen receptor positive breast cancer and could conceivably have etiologic significance.*

We recently suggested that there might be a relation between the development of breast cancer and impaired pineal function in women (*1*). There is evidence that the pineal product melatonin can decrease the number of breast tumors in dimethylbenz[*a*]anthracene (DMBA)-treated rats (*2*), and alter estrogen receptor (ER) concentration in vivo in ovariectomized rats and in vitro in human breast cancer cells (MCF-7) (*3, 4*). We undertook to examine pineal function by monitoring plasma melatonin concentrations over 24-hour periods in patients with breast cancer.

Biosynthesis of melatonin occurs in the pineal gland in a diurnal pattern that is reflected in the circulation by low concentrations during the day and high concentrations at night (*5*). This daily plasma profile has been essentially conserved over a wide range of vertebrate phylogeny (*6*), and in seasonally breeding species the daily melatonin rhythm may be the hormonal signal transducing environmental light information to the reproductive system (*7*). However, in nonseasonal breeders the physiologic role of this molecule remains unclear.

In nonseasonally breeding rats, daily afternoon melatonin injections significantly reduced the incidence of breast tumors in animals treated with a high dose (15 mg) of DMBA. Conversely, pinealectomy enhanced the incidence of tumors in rats given a low dose (7 mg) of DMBA. Assessment of circulating prolactin in DMBA-treated rats revealed significantly reduced levels in melatonin-treated rats, suggesting that melatonin may inhibit the induction of these prolactin-dependent tumors via prolactin suppression. Alternatively, melatonin may inhibit mammary tumor induction by its action on estrogen receptors. Oophorectomized animals are resistant to tumor induction by DMBA, but tumor induction can be restored by estrogen administration (*8*). These data suggest that daily treatment with melatonin or the presence of a normally functioning pineal gland can affect mammary tumorigenesis, perhaps by regulating the hormonal milieu of breast tissue or its intrinsic responsiveness to regulatory hormones.

Twenty women (aged 30 to 67 years) with clinical stage I or II breast cancer participated in a 24-hour blood sampling

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