Spectral Character of Sunlight Modulates Photosynthesis of Previtamin D3 and Its Photoisomers in Human Skin

Abstract. *The photosynthesis of previtamin D3 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 D3 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 D3. In comparison, when adjacent skin was exposed to simulated solar radiation, the maximum formation of previtamin D3 was about 20 percent. Major differences in the formation of lumisterol$_3$ and tachysterol$_3$ from previtamin D3 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 D3. Exposure to sunlight causes the photochemical transformation of 7-dehydrocholesterol (7-DHC to previtamin D3 (preD3) in human skin (1). The preD3 isomerizes by heat to vitamin D3 or by ultraviolet (UV) radiation to lumisterol$_3$ and tachysterol$_3$ (Fig. IA). The isomerizations of preD3, lumisterol$_3$, and tachysterol$_3$ are photoreversible reactions and therefore determine in part the yield of preD3 and, ultimately, of vitamin D3 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 preD3 and the photoisomerization of preD3 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 preD3, 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 7DHC and its photoproducts (1-3). Figure 1B illustrates an action spectrum thus obtained for the production of preD3 from 7-DHC in human epidermis. The optimum wavelengths for the production of preD3 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 preD3 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^o latitude in June at noon (2). With exposure to 295-nm radiation, the maximum possible conversion of 7-DHC to preD3 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 preD3 produced was only 15 to 20 percent of the original 7-DHC levels (Fig. 2A), and a quasiphotostationary state was established with tachysterol$_3$, lumisterol$_3$, and 7DHC 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 preD3 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 (1nM to I mM) and exposed to radiation of 295 ± 5 nm or to simulated solar radiation. The difference in the shape of the curves (Fig. 2A) for the percent conversion to preD3 from 7DHC in human epidermis or from 7DHC 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^o 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, preD3, lumisterol$_3$, and tachysterol$_3$ within this region. The forward reaction rate for any of the photoisomers (Fig. IA) is a product of the 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, preD3, lumisterol$_3$, and tachysterol$_3$ (Fig. 1C) demonstrate that 7-DHC and lumisterol3 (in both protic and aprotic solvents) show negligible absorption above 315 nm, whereas both preD3 and tachysterol$_3$ absorb radiation to at least 325 and 335 nm, respectively. Thus, the extinction coefficients for preD3 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 pre-D3 and tachysterol$_3$ significant. Even though the quantum yield for tachysterol$_3$ to preD3 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 preD3 to lumisterol$_3$, which accumulates because it is the least photoreactive isomer. To test this hypothesis, we exposed tachysterol and preD3 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 preD3 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 preD3 in human skin and the commercial production in vitro of previtamin D and previtamin D metabolites.

J. A. MacLAUGHLIN R. R. ANDERSON M. F. HOLICK

Vitamin D Laboratories, Endocrine Unit, Massachusetts General Hospital, Boston 02114, and Department Of Nutrition and Food Sciences, Massachusetts Institute of Technology, Cambridge 02139

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Fig. 1 (left). (A) The photochemical reaction in human epidermis of 7-DHC to preD3. The preD3 either thermally converts to vitamin D3 or photoisomerizes to lumisterol$_3$ and tachysterol$_3$. Quantum yields were adapted from (9). (B) The action spectrum of preD3 formation from 7-DHC in human epidermis (0) 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 preD3 from 7-DHC. (C) Ultraviolet absorption spectrums of (a) preD3, (b) tachysterol$_3$, (c) 7-DHC, and (d) lumisterol$_3$ isolated from human epidermis.

Fig. 2 (right). (A) Percent formation of preD3 from 7-DHC in human epidermis (----) or from crystalline 7-DHC (10 ~Lg/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 (A and A) in human epidermis after exposure to a range of doses of narrow-band radiation at 295 ± 5 nm (----) or to simulated solar radiation (1) or to (). 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.