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THE ROLE OF SUNLIGHT IN THE CUTANEOUS PRODUCTION OF VITAMIN D_3

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INTRODUCTION

Vitamin D is a unique vitamin/hormone whose origin dates back at least 0.5 billion years, when it was produced in ocean-dwelling phytoplankton while they were being exposed to sunlight (42b). There is mounting evidence that most plants and animals exposed to sunlight have the capacity to produce this secosterol. With the evolution of terrestrial vertebrates, vitamin D became important for the development and maintenance of the ossified skeleton. One

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of the major physiologic functions of vitamin D is to maintain blood calcium levels within the normal range in order to maintain neuromuscular function and bone mineralization. Vitamin D regulates calcium metabolism by its action on the intestine, where it increases the efficiency of intestinal calcium and phosphorus absorption and mobilizes calcium stores from the bone (48).

During the past few decades, intensive research on vitamin D has revealed that vitamin D is a hormone and not a vitamin. During exposure to sunlight provitamin D in the skin is photolyzed to previtamin D, a thermally labile intermediate that slowly converts to vitamin D. Once formed, vitamin D enters the circulation and is hydroxylated first in the liver to 25-hydroxyvitamin D (25-OH-D) and then to the kidney to form the biologically active form 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] (48). It is 1,25$(OH)_2$D that is responsible for enhancing the efficiency of intestinal absorption of dietary calcium and phosphorus. In addition this hormone is responsible for mobilizing stem cells to form osteoclasts—bone cells that are responsible for remodelling and mobilizing calcium stores from bone (48).

This chapter focuses on the recent advances in our understanding of how vitamin D is synthesized in the skin as a result of exposure to sunlight.

HISTORICAL REVIEW

The relationship of sunshine to health began to be appreciated at the turn of this century, but its roots began 200 years ago. As the number of city dwellers in Northern Europe increased, a disease identified by deformities of the skeleton (enlarged joints of the long bones and rib cage, curvature of the spine and thighs, enlargement of the head, and muscular weakness of the legs preventing support of body weight) was recognized as a major health problem. Characterized by Whistler, deBoot & Glisson (cited in 33), it was the clinical disease now known as rickets. Increasing industrialization saw a sharp rise in the incidence of rickets among the inhabitants of dim and densely populated streets.

The first advocate of sunlight as a prevention and cure was a Polish physician named Sniadecki (98), who in 1822 advised parents in Warsaw to take their rachitic children into the country, or at least carry them into the sun if possible. In 1889 the British Medical Association (84) reported a high incidence of rickets in industrialized towns, but not in urban regions of the British Isles. Observations throughout the British Empire led Palm, in 1890 (85), to reiterate Sniadecki's advice and to urge the measurement of the chemical activity of sunshine in cities and the use of sunbaths for therapy and prevention of rickets. In 1919 (49), Huldschinsky offered definitive proof of light's beneficial effects in the treatment of rickets. He cured four children with severe rickets by selectively exposing them to a mercury vapor quartz

lamp. He further showed that the effect was not localized since exposing one arm to the radiation resulted in healing of both arms. Sunlight as a cure for rickets was proven two years later by Hess & Unger (39) in New York; they exposed seven rachitic children to varying amounts of sunshine and saw radiologic evidence that the bone disease had resolved.

At the beginning of the twentieth century, many investigators were searching for the cause and cure of rickets, which had become a major health problem in the industrial cities of the world. A dietary antirachitic factor was first observed by Mellanby (72) in 1918 and initially thought to be vitamin A. Four years later this idea was refuted when the antirachitic factor present in cod liver oil was found to be a new substance and named vitamin D. In 1921 Powers et al (89) showed that cod liver oil and UV radiation had similar healing effects on rachitic rats, and the dual sources for antirachitic activity—diet and light—were established. During the next half century many workers contributed to the identification and understanding of the mechanisms of synthesis and biologic action of vitamin D: a historical account is given by Holick et al (46).

Today we know that there are two major forms of vitamin D: vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). The former results from the UV irradiation of a yeast sterol, ergosterol (provitamin D_2), the latter from irradiation of 7-dehydrocholesterol (provitamin D_3), a sterol that is naturally present in skin cells.

PHOTOCHEMISTRY OF PROVITAMIN D AND ITS PHOTOISOMERS

Provitamin D is a four-member ring steroid with two conjugated double bonds in the B ring at C5 and C7 ($\Delta^{5,7}$-diene), and with a side chain that identifies ergosterol or 7-dehydrocholesterol (Figure 1). The events leading to the formation of vitamin D (vitamin D without a subscript refers to either vitamin D_2 or vitamin D_3 or both) are now well known, primarily as a result of the work of Velluz and his colleagues (107). During irradiation of provitamin D with UV light the $\Delta^{5,7}$-diene sterol absorbs a quantum of energy that transforms it from the ground state to an excited singlet state. Ring opening at C9–C10 then yields a 6,7-*cis*-hexatriene derivative known as previtamin D. The previtamin can undergo a number of different reactions (Figure 2). A temperature-dependent isomerization with shifting of the double bonds followed by rotation about the single C6–C7 bond leads to vitamin D, a thermodynamically stable 5,6-*cis* isomer.

Upon further irradiation, previtamin D can absorb energy (between 240 and 320 nm) and can undergo one of three reversible photoreactions: (*a*) ring closure to its parent provitamin D; (*b*) ring closure to form the stereoisomer

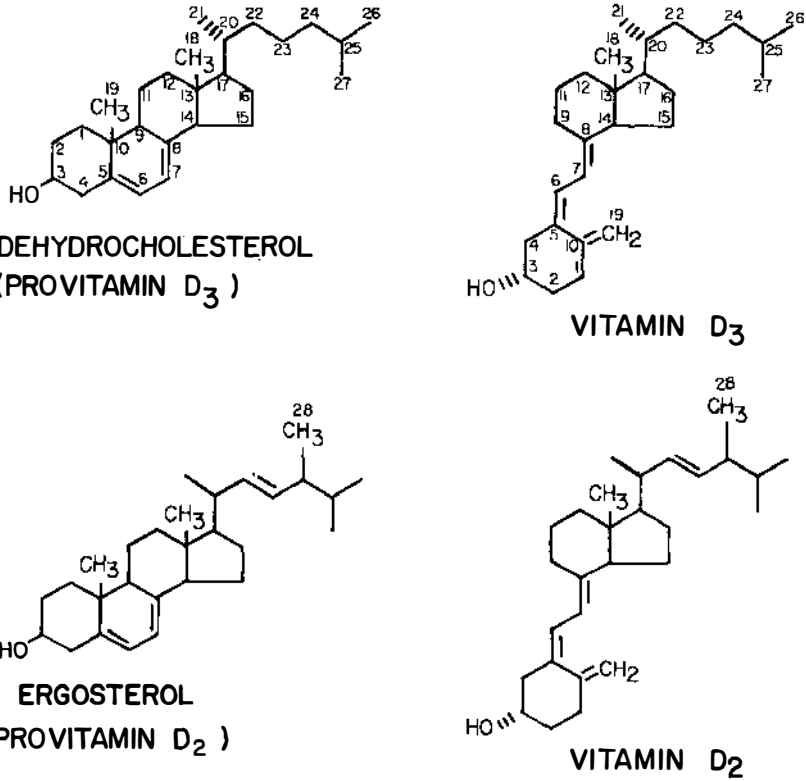

Figure 1 The structure of 7-dehydrocholesterol, ergosterol, vitamin D_2, and vitamin D_3. (Reproduced with permission from Ref. 46.)

lumisterol; or (*c*) isomerization to form a 6,7-*trans* isomer tachysterol. A quasi-photoequilibrium state is reached as irradiation is continued. In addition, there are a variety of photoproducts of previtamin D that can be produced by prolonged irradiation. At least 13 of these compounds, called toxisterols, have been identified by Jacobs et al (52). Work by Dauben & Bauman (20) has identified two photoproducts of vitamin D, known as suprasterols; a further four photoisomers of vitamin D were documented by Havinga (36).

FORMATION OF VITAMIN D_3 IN SKIN

Photobiology of Vitamin D_3

The first stage of vitamin D synthesis depends on the photoconversion of the 7-dehydrocholesterol (7-DHC) to previtamin D_3. 7-DHC is present in all layers of the skin, but the highest concentration per unit area of skin is found

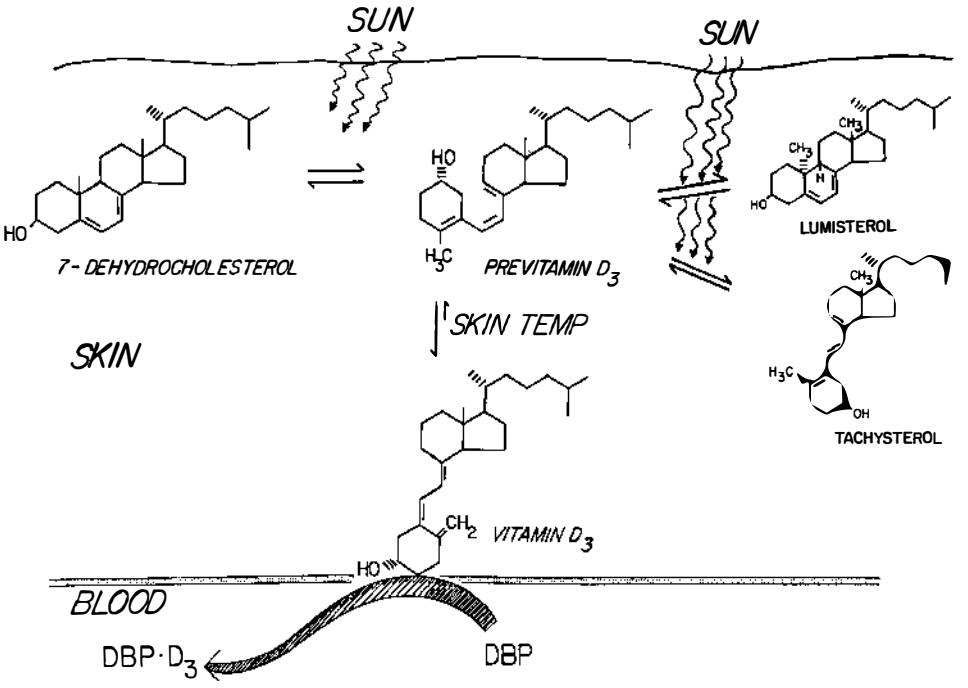

Figure 2 Diagrammatic representation of the formation of previtamin D_3 in skin during exposure to sunlight. The previtamin may then undergo further photoconversion to lumisterol and tachysterol, or thermal isomerization to vitamin D_3, which is then transported into the circulation bound to vitamin-D-binding protein (DBP). (Reproduced with permission from Ref. 45.)

in the stratum basale and stratum spinosum (44). These epidermal layers therefore have the greatest potential for previtamin D_3 synthesis, but the amount of photoconversion that can take place also depends on the quality and quantity of UV radiation reaching each layer of skin. Radiation of wavelengths in the UVB (280–320 nm) portion of the electromagnetic spectrum is required to convert 7-DHC to previtamin D_3. Holick et al (44) irradiated human skin with solar-simulated UV radiation and found that the production of previtamin D_3 was indeed maximized in the stratum basale and spinosum, though it occurred throughout the entire epidermis and to a small extent in the dermis. Once formed, the previtamin D_3 can undergo either photoconversion to lumisterol, tachysterol, and 7-DHC or a heat-induced isomerization to vitamin D_3 (Figure 2).

The temperature dependence of this thermal equilibrium reaction has been studied in vitro and in vivo by Holick et al (43, 47): At body temperature (37°C) 50% conversion occurs at 28 hours and it takes 4 days to reach equilibrium, with 80% of the previtamin converted to vitamin D_3. At 25°C,

50% conversion is achieved after 48 hours, whereas less than 2% conversion is observed after 7 days of incubation at −20°C. Therefore after an initial UV irradiation vitamin D synthesis can occur over a period of several days in the absence of sunlight.

Action Spectra for the Formation of Previtamin D_3 and Its Isomers

All the above photochemical reactions are dependent on the number and energy of the photons reaching each isomer over the waveband of its action spectrum. An action spectrum is generally defined by irradiating the object of study, be it a purified form of a parent compound or a living organism, with monochromatic radiation of different wavelengths and observing the efficiency of each wavelength at producing the desired result.

In the simplest case of a starting compound undergoing a complete nonreversible conversion to a single photoproduct, the reaction rate depends on the product of the quantum yield for the reaction and the number of photons absorbed. The latter quantity is in turn dependent on the intensity of the irradiation and the absorption cross section of the starting isomer (36).

For a reversible reaction with both isomers absorbing in the same spectral region, sufficient irradiation with light that can drive both forward and reverse reactions will produce an equilibrium between parent and daughter compounds. The balance of the equilibrium depends on the relative quantum yields and absorption cross sections of the two reactions.

In the case of previtamin D and its photoisomers (7-DHC, lumisterol, and tachysterol) the central compound (previtamin D_3) may undergo more than one reaction from irradiation with UVB quanta. The equilibrium condition is then a function of the irradiating spectrum and the absorption coefficients of the compounds involved. Figure 3 shows the ultraviolet absorption coefficients of each isomer and the consequences of irradiating a solution of 7-DHC with either monochromatic light or simulated sunlight, which produce different equilibrium mixtures. Under monochromatic irradiation the wavelength at which previtamin D_3 formation is maximized is 295±2 nm; tachysterol formation is most favored at 260 nm, while lumisterol peaks at 310 nm (46).

When studying photoeffects on living organisms, the additional factor of differential, wavelength-dependent interference from other constituents of the organism must be considered. For example, the conversion of 7-DHC to previtamin D_3 and then to lumisterol and tachysterol in human skin is affected by attenuation of light by the outer layers of the skin. Attenuation is greatest at the shorter wavelengths, those most effective for photolyzing 7-DHC, and the in vivo action spectrum becomes a convolution of the in vitro and attenuation spectra of the skin. The latter will, in turn, vary from person to person depending on age (thickness of the skin) and pigmentation (2). The result of

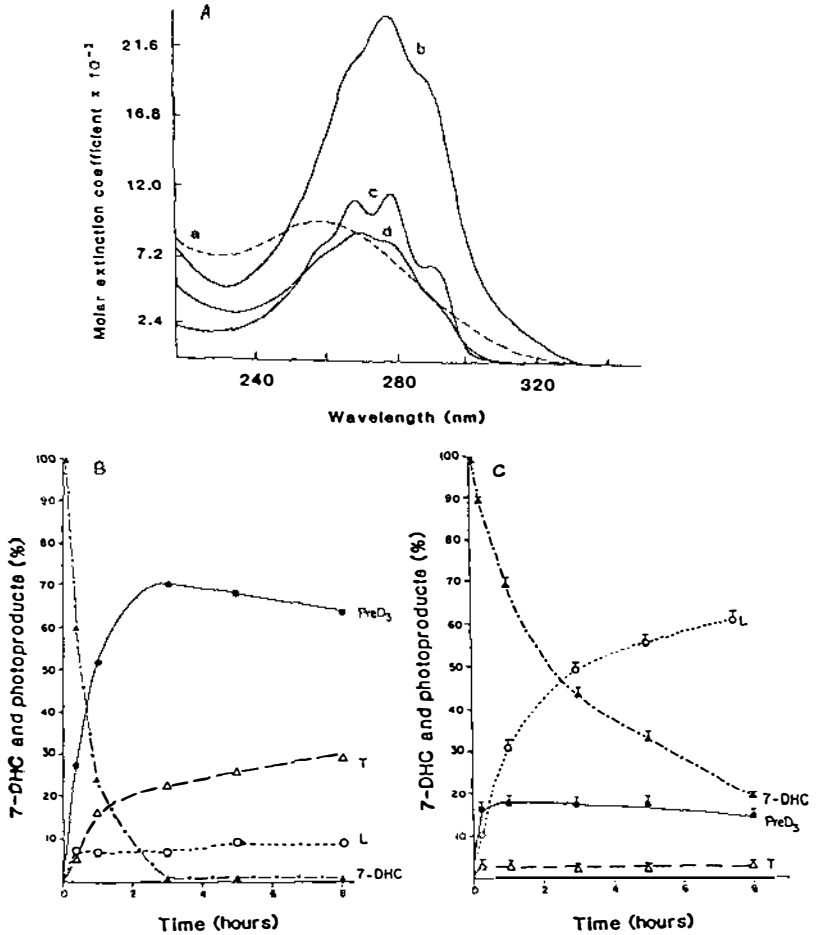

Figure 3 *Top:* Ultraviolet absorption spectrums of (*a*) previtamin D_3, (*b*) tachysterol, (*c*) 7-DHC, and (*d*) lumisterol isolated from human epidermis. (Reproduced with permission from Ref. 67.) *Bottom:* 7-DHC and photoproducts with increasing time of exposure to monochromatic 295 (±5) nm radiation *(left)* and tropical solar-simulated radiation *(right)*. (Reproduced with permission from Ref. 45.)

all these factors and the point of ultimate concern is the biological effectiveness of the radiation.

Bunker & Harris (9) determined that the most effective wavelength for curing rickets in rats was 297 nm, while Knudsen & Benford (55) showed 280 nm to have the greatest curative effect; 265, 289, 302, and 312 nm also have antirachitic properties. Bunker et al (10) fed irradiated 7-DHC to rachitic rats and concluded that 7-DHC irradiated at 297 nm had the greatest antirachitic activity; solutions irradiated at wavelengths longer than 313 nm showed no

activity. Later work by Kobayashi & Yasumara (56), working with ergosterol in solution, showed that irradiation with 295 nm radiation gave the maximum yield of potential vitamin D_2.

A further extension must be made from the idea of a simple action spectrum to the real-life effect of light: everyday illumination, whether natural or artificial, is unlikely to be monochromatic. The solar spectrum contains light from about 300 to 3000 nm (96), the intensity varying by many orders of magnitude over this spectral range. The waveband of concern for previtamin D synthesis is the narrow UVB waveband ($280<\lambda<320$ nm). This is at the short wavelength limit of the solar spectrum on the edge of the ozone absorption band where light is first able to penetrate through to the Earth's surface (95). The intensity of the incident radiation increases sharply with wavelength, while its effectiveness for photolyzing previtamin D_3 declines.

The consequences of the spectral character of sunlight on the photosynthesis of previtamin D_3 in human skin are discussed by MacLaughlin et al (67), who showed that under monochromatic irradiation at 295 ± 2 nm 65% of the 7-DHC in human skin could be converted to previtamin D_3. However, irradiation of adjacent skin to simulated solar irradiation produced a maximum of 20% previtamin D_3 (Figure 3, bottom) with a different pattern of accumulation for the other photoisomers: more lumisterol and less tachysterol than under the monochromatic irradiation (67).

Factors that Regulate the Cutaneous Production of Vitamin D_3

The concentration of 7-DHC and the quantity and quality of the UV radiation reaching the provitamin in the skin are the major factors governing the amount of previtamin D_3 produced in the skin. The wavelengths of radiation ($\lambda < 315$ nm) that initiate previtamin D_3 synthesis also come within the waveband absorbed by melanin. This led Loomis (65) to hypothesize that the evolution of racial distribution with latitude was due to vitamin D requirements: as people moved to higher latitudes pigmentation decreased to enable adequate cutaneous production of the vitamin. In recent years a better understanding of the photobiology and metabolism of vitamin D has shown that pigmentation is only one of the factors controlling the vitamin D status of a person.

At low levels of irradiation pigmentation is a limiting factor for previtamin D_3 synthesis because melanin is absorbing ultraviolet photons in competition with 7-DHC. Increasing melanin in human skin requires a longer exposure to, or higher intensity of, UV radiation to maximize previtamin D_3 formation, but given sufficient irradiation pigmentation does not prevent previtamin D_3 synthesis (45). Lo, Paris & Holick (64) showed that the increase in serum vitamin D levels after whole-body exposure to 1.5 MED (minimal erythema dose) was the same in both Asians and Caucasians, although the Asians in the

study received more total irradiation than the Caucasians because of their need for more exposure to produce 1 MED. A single exposure to UVB radiation, from 1 to 4 MED (280–320 nm), has been shown to raise serum vitamin D levels by an amount that increases with increasing dose of UV radiation (1). The same irradiations produced smaller increases in serum 25-OH-D, and did not show the same marked dose dependence, which suggests a metabolic control on 25-OH-D output from the liver. Only vitamin-D-deficient patients exhibited a significant rise in serum $1,25(OH)_2D_3$ after a single exposure to UVB, which supports the idea that the synthesis of this metabolite is strictly regulated in vitamin-D-sufficient humans.

Regardless of the amount of pigmentation in the skin (Caucasian, Asian, or African) exposed to UV radiation, the previtamin D_3 produced does not increase above a maximum value of about 15% of the original 7-DHC concentration (45). Further exposure to sunlight simply causes the photoisomerization of previtamin D_3 to lumisterol and tachysterol (45). With the slow heat isomerization of previtamin D_3 to vitamin D_3, the quasi-equilibrium between previtamin D_3 and its photoisomers is upset. If sufficient time has elapsed since the initial irradiation for a reduction in previtamin D_3 to occur, further irradiation may result in photoconversion of 7-DHC, lumisterol, or tachysterol to their parent compound. Failing this, the two photoisomers are probably lost during natural sloughing off of the outer layers of the skin. Although biologically inert in themselves (67), the photochemical conversion of previtamin D_3 to lumisterol and tachysterol imposes an important limitation on the amount of previtamin D_3 that can accumulate in human skin (Figure 3, bottom).

Aging also has an effect on cutaneous previtamin D_3 synthesis as it reduces the amount of 7-DHC in the skin. Most vitamin D_3 is produced in the epidermis (44), which does not significantly decrease in mass with time, despite a linear decrease of dermal skin thickness with age above 20 years (103). However, there is a linear decrease in 7-DHC concentration in the skin with age (68) (Figure 4); in a person 80 years old, the concentration of 7-DHC in the epidermis is only half that of a 20 year old. When skin samples from young and elderly subjects were exposed to simulated solar sunlight they showed that the amount of previtamin D_3 generated in young skin was more than twice that produced in skin from an elderly subject (68).

An artificial regulator of cutaneous production of previtamin D_3 is the use of sunscreens. Sunscreens are intended to prevent the damaging effects of the sun's rays by blocking or reducing the UVB part of the spectrum reaching the surface of the skin. In doing so they also reduce or prevent the photoconversion of 7-DHC to previtamin D_3 since this beneficial reaction makes use of the same UVB wavelengths that are responsible for erythema. A sunscreen with a sun protection factor of 8 has been shown to prevent the production of previtamin D_3 in human skin both in vivo and in vitro (70).

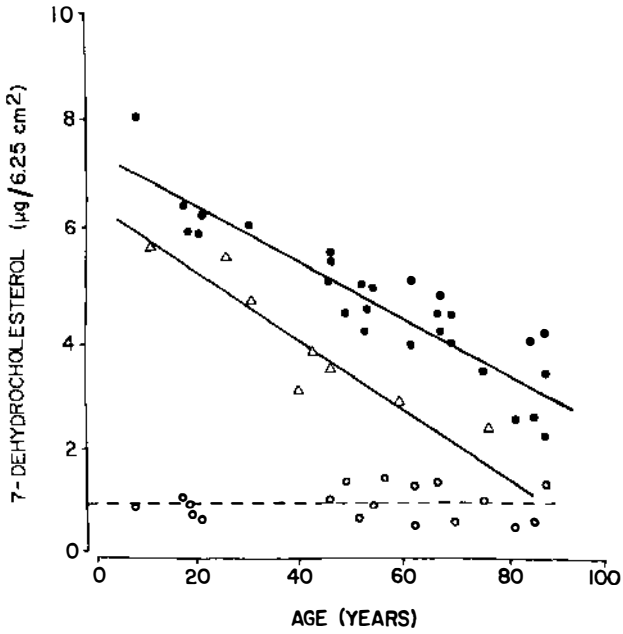

Figure 4 Effect of aging on 7-DHC concentrations in human skin. Concentrations of 7-DHC per unit area of human epidermis *(solid circles)*, straum basale *(triangles)*, and dermis *(open circles)*. (Reproduced with permission from Ref. 68.)

The aforementioned regulators are all endogenous or topically applied and control the effectiveness of the incident irradiation. However, the solar irradiation reaching the skin is also important. Its intensity and spectral character are functions of both latitude and season, and their changes are the premise upon which Loomis' theory of evolution (65) was based.

The waveband of interest for previtamin D_3 synthesis is in the UVB. No part of the solar spectrum with wavelengths shorter than 290 nm reaches the Earth's surface, since they are absorbed in the upper atmosphere, primarily by oxygen (λ <200 nm) and ozone. The absorption coefficient of ozone decreases as wavelength increases into the UVB waveband, and the amount of solar UVB radiation reaching the biosphere is a function of wavelength and the amount of ozone encountered en route (8). This latter quantity depends on the concentration of ozone in the atmosphere (usually denoted as the amount in a vertical column when reduced to standard temperature and pressure) and the pathlength of light through the atmosphere. Atmospheric concentration has a latitudinal dependence, and superimposed on this are a number of periodic variations: annual, biennial, and 22-year cycles (12, 110). Distance that light has to travel through the atmosphere is a function of the solar zenith angle and depends on latitude, season, and time of day. Further atmospheric

attenuation comes from Rayleigh scattering, which is proportional to λ^{-4} (96). Thus as wavelength gets shorter in the UVB part of the spectrum both absorption and scattering increase rapidly and intensity declines sharply.

Comprehensive spectral measurements of incident solar UVB radiation are not available worldwide, although they have been made at a number of locations (6, 108) and more are being made as technology improves. With the advent of satellites, ozone measurements have also improved (3, 110) both in quality and coverage, and together with better understanding of atmospheric chemistry this has enabled global models of incident UVB to be formulated (32, 51, 75). Thus, theoretically, this information, coupled with the action spectrum for the conversion of 7-DHC to previtamin D_3, allows an approximate estimate of the solar effectiveness for cutaneous vitamin D_3 production around the world. However, the solar spectrum changes by up to two orders of magnitude from summer to winter (7), with the short wavelength cutoff also changing by season and latitude. Any inaccuracy in the spectrum used could result in errors in the assumed ability of the skin to synthesize vitamin D_3 at a given location.

We have developed a model in our laboratory to assess the seasonal and latitudinal efficacy of the sun, or any light source, for previtamin D_3 synthesis (submitted for publication). Quartz test tubes containing ^{3}H-7-DHC in methanol are exposed to sunlight on clear days throughout the year for a three-hour period from 11:30 to 14:30 Eastern Standard Time. Aliquots taken each hour and analyzed by high-performance liquid chromatography (HPLC) using a UV detector (254 nm) and an in-line radioactivity detector show the photoconversion of 7-DHC to previtamin D_3 and its photoisomers. Figure 5 shows the seasonal conversion of 7-DHC to previtamin D_3 in Boston (42.2°N) and Edmonton (52°N). Our model has also been used at other locations: Los Angeles (34°N), Puerto Rico (18°N), Warsaw (51°N), and Pisa (42°N). It can be seen that in Boston there is no photolysis of 7-DHC during the winter months of November through February. Thereafter the amount of previtamin D_3 detected increases to a maximum in June and July, before declining to zero again as solar altitude decreases. In Edmonton, 10° further north, the unproductive months of the year for vitamin D_3 synthesis are extended by one month at either end, from October through March. By contrast, to the south in Los Angeles and Puerto Rico there was conversion of 7-DHC to previtamin D_3 in January, and we see a time-latitude relation for previtamin D_3 synthesis.

Once previtamin D_3 is formed, further photochemistry is possible and the irradiating spectrum governs the nature of the quasi-photoequilibrium state between previtamin D_3 and its isomers at any given time. Since the experimental solution is in a quartz tube that allows for full transmission of UVB radiation, the model gives the maximum conversion that could occur at any time. The amount of previtamin D_3 in the mixture does not increase in-

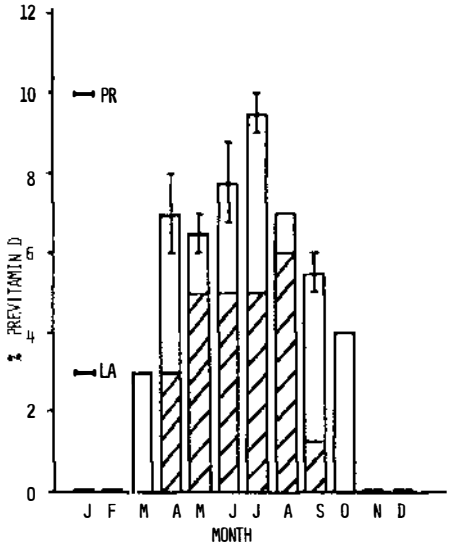

Figure 5 Annual change in previtamin D_3 production as a percentage of the starting solution of 7-DHC exposed to sunlight for one hour at noontime. Exposure in Boston *(clear bars)*, Edmonton *(cross-hatched bars)*, Los Angeles (LA), and Puerto Rico (PR). Where conversion was different for the years 1986 and 1987, the average value is plotted and bars indicate the two measured levels of conversion.

definitely but plateaus at about 12% of the original amount of 7-DHC, and it is the amount of lumisterol that then continues to increase. The previtamin D_3 maximum of 12% is less than that seen in the laboratory under simulated tropical sunlight (67) for two reasons: Boston sunlight is not tropical, and it is not constant but declines in intensity, with the spectrum also shifting away from the shorter wavelengths as the exposure proceeds. A small amount of tachysterol was observed in Boston in June and July, of the order of 2% of the original 7-DHC concentration. The small amount of this isomer is consistent with the results of exposing a starting solution of tachysterol to sunlight: there is rapid conversion to previtamin D_3 and thence to its other photoisomers in preference to tachysterol. Such data prove our model to be an effective and direct method of assessing seasonal and latitudinal changes in the solar-induced photochemistry of vitamin D_3.

To validate and relate our model to what occurs in human skin, recently excised human skin was exposed to sunlight at the same time as the solutions in February, April, June, and October. Results concur with those from our model in a qualitative sense: no conversion was observed in February, maximum conversion was seen in June. Quantitatively the amount of conversion in human skin, under both natural and simulated sunlight, was about half that seen in our test tube model. This reduction is due to the attenuating properties of the outermost layers of skin (2).

Photodegradation of Vitamin D_3

The cutaneous production of vitamin D_3 is well documented, as are the overirradiation products of vitamin D_3 when exposed to high levels of UV radiation (36, 57). However, the effect of sunlight on vitamin D_3 in skin had not been considered until recently.

The test tube model developed to study the solar-induced photoconversion of 7-DHC has also been used to investigate the effect of sunlight on vitamin D_3 itself. ^{3}H-vitamin D_3 in methanol was exposed to sunlight throughout the year in the same manner as 7-DHC, and the aliquots analyzed in the same way by HPLC. We found that throughout the year the vitamin D_3 was degraded by sunlight; a three-hour exposure resulted in a 30% reduction in vitamin D_3 in December, and over 90% reduction in June. The three main photoproducts of this photolysis were identified as 5,6-transvitamin D_3 and the suprasterols I and II (109). It is not known whether these products have any biological significance.

The relevance of this finding to cutaneous concentrations of vitamin D_3 in vivo was tested by irradiating freshly excised skin to produce previtamin D_3, incubating it for 24 hours, and then reexposing the skin to either natural or simulated sunlight. The vitamin D_3 in the skin was degraded by 25–60% depending on time of year and method of the second irradiation.

Measurements of the action spectrum for the breakdown of vitamin D_3 showed that the active wavelengths extended from the UVC (200–280 nm) to 335 nm. This supports the results from our test tube model: vitamin D_3 is photolabile at wavelengths (315–335 nm) longer than those that initiate the formation of previtamin D_3 (λ <315 nm), and as these wavelengths are always present in the solar spectrum in Boston, degradation is observed throughout the year.

Artificial Light Sources and Vitamin D

Natural environmental stimulation of previtamin D synthesis changes with time and latitude, the "vitamin D season" becoming shorter at higher latitudes, and in winter the time spent indoors under artificial lighting is likely to exceed that spent in the sun. Window glass does not transmit radiation of wavelengths less than 334 nm (38)—therefore natural lighting that is transmitted indoors is of no benefit for cutaneous vitamin D_3 production. Domestic artificial lighting may contain radiation of UVB wavelengths, and some lights, especially fluorescent tubes, do emit short wavelength radiation (λ <280 nm). However, the glass or plastic fixtures in which they are mounted are usually designed to shield any potentially damaging radiation below 320 nm (16, 53, 71). As the wavelengths of radiation responsible for 7-DHC photolysis are also those that produce erythema and can lead to skin cancer, care must be taken to limit the UVB output of any devices intended for long-term use. Efforts are being made by the lighting industry to match

artificial lights more closely to the spectrum of the sun, as in the commercially available Vita-Lite® fluorescent tubes (Duro-Test Corp., New Jersey). These lights, which emit 5% of their total radiant output at wavelengths between 290 and 380 nm, showed no positive effects on the levels of plasma 25-OH-D for hospital patients exposed to the lights at normal illumination levels for a period of one year (23). However, Neer et al (77) showed that elderly men receiving no exposure to natural sunlight or dietary vitamin D increased their calcium absorption when exposed to high-intensity Vita-Lite for 8 hours per day. No measurements of vitamin D status were made during this study.

On a shorter time scale, commercially available sunlamps have been tested for their ability to promote vitamin D synthesis (21) and proved to be effective. However, they also caused other adverse skin reactions, such as erythema, over the time period required; a result of the shorter ($\lambda < 290$ nm) and more damaging wavelengths that the spectra of many sunlamps contain when compared with the sun (74). Controlled UV chambers such as those used for phototherapy have proved to be a safe and efficient way of preventing vitamin D deficiency in the elderly when used to give a weekly dose of UV radiation (106). A UVB light box (National Biologic Radiation Corp.) of the type used for phototherapy was tested for its ability to promote previtamin D_3 synthesis using our test tube model. 7-DHC converted to previtamin D_3 at the rate of 1% conversion per mJ of irradiation. At 30 mJ of irradiation (about 1 MED for a Caucasian) there was a 30% conversion to previtamin D_3 with no lumisterol or tachysterol present in the solution. This is different from what can be expected after exposure to sunlight with its maximum of 15% conversion to previtamin D_3, and it reiterates the importance of the irradiating spectrum for the efficiency of previtamin D_3 formation.

EPIDEMIOLOGY OF VITAMIN D

Vitamin D Deficiency in Children and Adults

The childhood disease rickets with its characteristic bone deformations is a well-recognized result of vitamin D deficiency during the growth and developmental stage of the skeleton. Once growth ceases, the bones remain the same length but are constantly being remodelled. Vitamin D is still necessary for this remodelling process. A lack of the vitamin in adults causes defects in the mineralization of the bone collagen matrix, a disease known as osteomalacia (28). A more common adult bone disorder is osteoporosis; skeletal weakening due to loss of both bone collagen matrix and bone mineral. There are many contributory causes of this disease, including a lack of vitamin D, and no therapy has proven to be perfectly safe (78), so prevention is of prime importance. The loss of bone mass associated with these skeletal disorders brings a greatly increased risk of fractures, particularly of the hip, wrist, and

spine. Attendant problems come with the possibilities of complications and permanent disability, and the financial burden that acute and chronic care of fractures entails (18).

There are a number of factors that singly or in combination can result in the symptoms of a deficiency of vitamin D: a lack of dietary intake, impaired intestinal absorption, lack of sunlight exposure, reduced cutaneous production due to aging, melanization, sunscreen use or other causes (42), impaired metabolism of vitamin D to its biologically active form $1,25(OH)_2D_3$, and lack of responsiveness of the target tissue to the active metabolite (100).

The traditional victims of rickets were the undernourished, light-starved children of industrialized inner cities (46). Vitamin D is naturally present in few foods (fish liver oil, eggs, liver, and milk) but in some countries milk (US), some cereals, and margarine (UK) are fortified with vitamin D_2 or D_3 (69, 86). With the advent of food fortification and an improvement in air pollution and living conditions, rickets has become a rare disease among the indigenous population of Europe and the US, but it is still seen among the children of immigrants, particularly Asians in Europe (104).

A combination of factors results in the vitamin D deficiency of Asian children (30, 101). Their diet is low in fortified foodstuffs but high in fiber [which may increase the rate of turnover of 25-OH-D (4)] and phytic acid [which decreases the bioavailability of dietary calcium (92, 94)]. Exposure to UV radiation is low because of the move to higher latitudes (101) and for social and religious reasons; furthermore the absorption of UVB radiation by melanin pigmentation exacerbates this and reduces cutaneous synthesis of vitamin D_3 (13). None of these factors alone need cause deficiency: Linhares et al (62) reported high levels of 25-OH-D in both well- and undernourished Brazilian children, which indicates that diet is not important when there is sufficient UVB exposure to compensate for pigmentation. Turkish immigrants in Germany with dietary habits different from those of Asians also showed signs of vitamin D deficiency (82). Thus while the Asian diet may contribute to a susceptibility to rickets, it is a melanin-induced decrease in the exposure of 7-DHC in the skin to sunlight that is more important. Pigmentation is only limiting over short exposure periods. With sufficient exposure time and intensity of irradiation (increasing with increasing melanin content of the skin), the same circulating concentrations of vitamin D_3 can be reached in people of European, Asian, and African origin (45).

As the incidence of rickets has declined with improved standards of living, the occurrence of the adult bone disorders of vitamin D deficiency has increased as the elderly population expands. The reasons for this are multifactorial. Dietary supply of the vitamin is often low, even in the countries where foods are fortified. In the US the consumption of one quart of milk would provide the recommended daily allowance of the vitamin (400 IU) (86)

but many elderly drink little milk because they (*a*) consider it unnecessary, (*b*) dislike it, or (*c*) have a lactase deficiency and develop gastrointestinal distress when they drink it (41). Another consideration is an age-related decline in intestinal absorption of the dietary form of the vitamin, yet Holick (41) recently reported that when tested for vitamin D absorption healthy elderly patients showed no major reduction of intestinal absorption compared to young adults. In contrast to healthy subjects, whose circulating vitamin D_2 was increased 12 hours after its administration, patients with a variety of malabsorption syndromes showed no increase in circulating vitamin D_2 after receiving the same oral dose as normal subjects (63).

If dietary intake is low, an adequate supply of vitamin D must be provided by cutaneous synthesis, a source that is jeopardized by various facets of aging. A decrease in mobility and (at high latitudes) cold intolerance reduce the time that many elderly spend outdoors exposed to sunlight. The reduced UVB radiation reaching the skin is also rendered less effective by age-related changes in the skin. Early observations by Lester et al (61) showed that summer levels of circulating 25-OH-D were lower in active elderly subjects than in their young counterparts, a finding consistent with the observation that provitamin D concentrations decrease in the epidermis with age (68).

Even with an adequate supply of vitamin D, metabolic conversion to its active form $1,25(OH)_2D_3$ is still necessary before the vitamin D can function physiologically to increase intestinal calcium absorption and remodel bone. If the kidney does not respond to stimuli and produce the active metabolite of vitamin D when required (97), then the body will take the calcium that it needs from mineral stores in the bone rather than from the diet through intestinal absorption. Doses of $1,25(OH)_2D_3$ have been shown to enhance the efficiency of calcium absorption to the same extent in both young and elderly subjects (79), and the response of the intestine to the active vitamin D metabolite is not affected by aging. However, the ability of the intestine to increase efficiency of absorption when challenged with a calcium-deficient diet decreases with age (50) and this may be due to an acquired renal defect in the metabolism of 25-OH-D to $1,25(OH)_2D$. The levels of $1,25(OH)_2D_3$ in osteoporotic women have been reported to be lower than those of age-matched controls (93), although they are still within the normal range.

The preceding discussion reveals that vitamin D deficiency may arise from a number of factors acting at different stages along the route from source to effective action of the vitamin. This poses the problem of identifying vitamin D deficiency in a clinical setting. The most commonly used measure of vitamin D status is the circulating concentration of 25-OH-D, for which there are now routine radioligand binding protein assays (34, 54). The normal range for 25-OH-D in our laboratory is 8–55 ng/ml, and assays in other laboratories have established similar ranges. Values below 10 ng/ml usually indicate vitamin D deficiency (42, 73). Circulating levels of $1,25(OH)_2D$ are about

1000 times less than those of 25-OH-D and require a more rigorous assay procedure (14). In our laboratory, normal range for circulating concentrations of this metabolite is 26–65 pg/ml.

Environmental Factors

Sunlight and diet are well established as the two sources of vitamin D, but the relative importance of each is less well defined, changing by location and social situation. Once it enters the circulation, either directly from the skin or by absorption after ingestion, vitamin D follows the same metabolic pathway regardless of its origin, and the question of supply mechanism is only important up to this point.

There can be little doubt that before the era of vitamin supplementation and food fortification, sunlight was the main provider of vitamin D for much of the world's population. For those races living in climates unfavorable for sunlight exposure, e.g. Eskimos, a diet naturally rich in fatty fish compensated for the lack of sun. Nearer the equator, the increasing intensity and extension to shorter wavelengths of the solar spectrum enabled cutaneous synthesis to occur, first on a seasonal basis, and then, at still lower latitudes, year round. The increase in skin pigmentation toward the equator was offset by the increase in solar intensity; melanin was not a limiting factor for vitamin D synthesis. Today, racial integration around the world, increased life expectancy, and indoor occupations have all upset this balance for some sections of the population. Asian and African people in Northern climes, the infirm elderly, and those employed in occupations such as submarine crews (90) are exposed to less sunlight than they would be in the circumstances under which they evolved. A large number of studies have addressed the relative merits of sunlight exposure and diet in supplying the vitamin D requirements of different populations.

The latitudinal effect on sunlight exposure has already been discussed, and as sun exposure decreases, for whatever reason, below that required to produce adequate vitamin D in the skin, dietary vitamin D becomes more important. Seasonal variation of plasma 25-OH-D was first observed by Stamp & Round (102) working with healthy white subjects in Britain, although the high-spring, low-autumn incidence of rickets had been noticed many years previously (37). High autumn values of plasma 25-OH-D_3 declined during the winter months and then rose again throughout the spring and summer to approach previous autumn values by August. Seasonal variations in plasma 25-OH-D have since been observed by a number of workers studying healthy young people in Britain and Scandinavia (5, 24, 58, 88). At high latitudes when synthesis of vitamin D_3 is not possible during the winter months, deficiency status is avoided by using stores of vitamin D built up in body fat tissues during the summer months. Provided summer sunlight exposure has been adequate, there should be sufficient reservoirs to maintain

healthy values until the following spring, and supplementation is not needed.

Other studies in Denmark and Britain have shown a correlation between the 25-OH-D levels and dietary vitamin D intake of healthy young adults (60), although mean levels were higher among Danish subjects taking supplements. Lund & Sorensen (66) showed a positive correlation between sun exposure and plasma 25-OH-D levels in Danes of all ages who did not take regular vitamin supplements. Subjects taking approximately 200 IU of vitamin D per day maintained a higher annual level of plasma 25-OH-D and did not show the same marked seasonal variation. However Poskitt et al (88) in Britain found that for healthy young people the most important long-term source of the vitamin was through the action of UV light on the skin. Giving vitamin D orally at twice the recommended daily intake caused only a slight increase in plasma 25-OH-D concentration, which suggests that the average British intake of 100 IU per day is an insufficient source of vitamin D.

Vitamin D levels in the elderly are low compared with those of younger subjects, even for healthy people (17, 59, 83, 102). The annual range of plasma 25-OH-D concentrations for office workers and children in Britain is 8–36 ng/ml (5, 88), while for healthy elderly the seasonal range has been reported as 8–14 ng/ml (61) and 10–19 ng/ml (19). Because the elderly do not approach the same maximum summertime levels as the young, they are more prone to deficiency levels of vitamin D during the winter months. Devgun et al (22) showed that differences in plasma 25-OH-D levels from year to year are associated with annual differences in UVB radiation: a low summer exposure will result in lower winter plasma 25-OH-D levels than for a winter following high summer exposure.

Lamberg-Allardt (58) reported observations from four populations in Finland: long-stay geriatrics, residents of old people's homes, healthy elderly, and young adults. All four groups showed seasonal variations of plasma 25-OH-D concentration, the amplitude of variation decreasing with activity. The group with the lowest 25-OH-D levels at all times, and the smallest variation, were the long-stay patients who had only brief outdoor exposure during the summer. This group was also the only one to show any correlation between dietary vitamin D intake and plasma concentration. Toss et al (105) also observed higher concentrations among healthy elderly than those in institutions. Nayal et al (76) found a correlation between diet and 25-OH-D levels in British patients in a geriatric unit when sunlight exposure was very low. These results suggest that in Northern Europe environmental UVB radiation is sufficient to maintain a healthy vitamin D status if exposure times approach those of an active healthy adult. When exposure is very brief, some dietary supplementation may be necessary.

For many years vitamin D deficiency among the elderly in the US was not considered a problem. The land mass is further south than Britain so the

ambient UVB is greater and milk is fortified with vitamin D; it was assumed therefore that vitamin D would be adequate from one source or another. However, a study in Albuquerque, New Mexico (15), showed circulating 25-OH-D levels of free-living elderly to be about half that of young adults, with 15% of the elderly identified as vitamin D deficient. A study of 373 healthy women in Iowa (99) aged 20–80 years showed no evidence of vitamin D deficiency in the population, although the older women had lower 25-OH-D levels than the young. In Boston, 40% of hip fracture cases presenting to one hospital had low or undetectable concentrations of 25-OH-D (26). A recent study of ours (in preparation) of the elderly in different mobility states in Boston showed a correlation between UVB exposure, due to both season and mobility, and circulating 25-OH-D levels. For those subjects not taking supplements and not independently mobile, up to 50% became vitamin D deficient during the winter months. Subjects taking a daily supplement of 400 IU vitamin D all remained within the normal range of plasma 25-OH-D levels throughout the year, but did show a seasonal variation related to sunlight exposure.

No single recommendation for adequate sunlight exposure can be made; any such statement must always be qualified with time and place. It is estimated (41) that for the elderly white population of Boston, exposing hands face and arms on a clear summer day for 10–15 minutes (depending on pigmentation), 2–3 times a week, should be sufficient to maintain a healthy vitamin D status. Application of a sunscreen after this initial beneficial exposure would then prevent any harmful effects from the sun's rays.

When it is not possible to take advantage of this natural source of vitamin D, alternatives are available. Toss et al (106) found that UV lamp treatment given as a weekly dose in controlled UV chambers was a safe way of preventing D deficiency in the elderly, but was very time consuming. The simplest means to avoid deficiency is through the diet, either by drinking a quart of vitamin-D-fortified milk a day (in the US) or by taking a vitamin supplement containing 400 IU of vitamin D. The minimum daily requirement for health is estimated at 2.5–10 μg (30), that is 100 IU (as in Britain) to 400 IU (as in the US). The latter has proved from our study to be adequate supplementation; the former dose may be too low to be fully effective (88). While this remedy is simple, care must be taken not to exceed the required dose. Prolonged oral doses of large quantities of vitamin D can be harmful (25), and intakes of over 2000 μg (80,000 IU) per day are known to be very toxic (29).

CONCLUSION

It is natural exposure to sunlight that is responsible for maintaining adequate vitamin D nutrition for most of the population of the world. This fact was first appreciated at the turn of this century, when it was finally shown that lack of

exposure to sunlight was responsible for the development of rickets in the children of industrialized countries. During the past 15 years significant progress has been made in our understanding of the basic photobiologic process that results in the production of vitamin D_3 in the skin. Contrary to popular belief, exposure to sunlight does not result in the direct production of vitamin D_3. Instead, a thermally labile precursor known as previtamin D_3 results from the photolysis of provitamin D in the epidermis. Once formed, previtamin D_3, in the absence of further exposure to sunlight, is converted over a period of 2–3 days to vitamin D_3. It has long been assumed that a short exposure to sunlight resulted in a limited production of vitamin D_3, while a prolonged exposure resulted in an increased production of vitamin D_3. Although this is true to a certain degree, there is now firm evidence that prolonged exposure to sunlight results in the photodegradation of previtamin D_3 to biologically inert photoproducts. Furthermore repeated exposure to sunlight day after day does not necessarily increase the total cutaneous production of vitamin D_3 because once formed vitamin D_3 is also photodegraded. Overall it is photochemistry that is most important for regulating the cutaneous production of vitamin D_3 and it is this phenomenon that prevents vitamin D intoxication in sun worshippers, lifeguards, and people who are exposed to sunlight every day because of their daily activities.

When exposure to sunlight is not adequate to maintain vitamin D status, then it is essential to obtain vitamin D from a dietary source. Unlike exposure to sunlight, which regulates the total production of vitamin D_3, the oral ingestion of vitamin D in large quantities can result in vitamin D intoxication. There is no evidence to date that the biologic effects of vitamin D that is produced in the skin on calcium and bone metabolism are any different from those produced by vitamin D that is ingested. However, it should be noted that recently a new vitamin D was isolated from mammalian skin and was identified as 24-dehydrovitamin D_3 (40). Although no specific biologic function has been observed for this new vitamin D, it has been demonstrated in vitro that 24-dehydrovitamin D_3 is extremely effective in inhibiting the 25-hydroxylation of vitamin D (8b). This may be another mechanism whereby excessive exposure to sunlight does not result in vitamin D intoxication because the metabolism of vitamin D to its first intermediate is blocked by 24-dehydrovitamin D.

There is mounting evidence that our elderly population, especially those who are institutionalized or are infirm, have a significant incidence of vitamin D deficiency. Some have suggested that vitamin D deficiency in the elderly throughout the industrialized nations is an unrecognized epidemic that requires attention (26). Adult rickets in combination with osteoporosis may very well significantly increase the risk of hip fracture. The simple remedy is to advise the elderly either to go outdoors and take advantage of the beneficial

effect of exposure to sunlight for short periods of time or to take some dietary source of vitamin D, either by drinking fortified milk or taking a vitamin D supplement that contains 400 IU of vitamin D. Thus, although prolonged exposure to sunlight can increase the risk of skin cancer several decades later and can cause photoaging of the skin, for the elderly it must be appreciated that the immediate effect of lack of sunlight exposure can cause vitamin D deficiency bone disease that ultimately could result in a higher incidence of fracture.

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