Photosynthesis, Metabolism, and Biologic Actions of Vitamin D

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HISTORICAL INTRODUCTION

Vitamin D is a most interesting seco-sterol that has its origin dating back at least 0.5 billion years ago when it was produced in ocean-dwelling plankton (1). Although it is not known what its physiologic function was in these lower life forms, it appears that vitamin D became extremely important in the evolution of animals with ossified endoskeletons. In terms of human history, the role of sunlight and vitamin D became important at the beginning of the industrial revolution. As people began to congregate into cities and their children played in crowded, sunless alleyways (Fig. 1), the children developed a severe bone disease that was first appreciated in the mid-seventeenth century by Whistler, Glisson, and DeBoot (Fig. 2) (2,3). 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 nineteenth century, autopsy studies done in Leiden (The Netherlands), suggested that approximately 90% of the children raised in this crowded and polluted city had the disease. This disease had devastating consequences for young women who often had a deformed pelvis that resulted in very high incidences of infant and maternal morbidity and mortality. It was this single factor that led to the increased use of cesarean sectioning for delivery of children from rachitic mothers.

As early as 1822, Sniadecki observed that children living in Warsaw had a much higher incidence of the disease compared to children living in rural areas. These observations prompted him to conclude that exposure to sunlight was the most important factor in the prevention and cure of rickets (4). Almost 70 years later Palm (5) reported his epidemiologic survey that included clinical observations from a number of physicians throughout the British Empire and the Orient. Based on his observation that rickets was rare in children who lived in squalor in the cities of Japan, China, and India whereas children of the middle class and poor who lived in industrialized cities in the British Isles had a high incidence of this disease, he concluded that the common denominator was sunlight. He urged the systematic use of sunbathing to prevent and cure rickets (5). However, it was inconceivable at that time
for scientists and physicians to accept such a simple remedy for such a devastating bone disease. As a result, another 30 years would pass before Huldshinsky un-equivocally demonstrated that exposure to radiation from a mercury arc lamp could cure rickets (6). Two years later Hess and Unger exposed seven rachitic children on a roof of a New York City hospital to varying periods of sunshine and reported that x-ray examination showed marked improvement of rickets in each child as evidenced by calcification of the epiphyses (7).

Simultaneously, Mellanby reported that he could produce rickets in dogs by feeding them oatmeal and could cure the disease by adding cod-liver to their diet (8). Indeed, it was a common folklore practice on the coast lines of the British Isles and the Scandinavian countries to use fish liver oils to prevent and cure this bone de-forming disease. At first it was thought that the antirachitic activity in cod-liver oil was due to vitamin A. However, when the vitamin A activity was destroyed by heat and oxidation the cod-liver oil continued to have antirachitic activity (9). As a result of these observations, it was concluded that there was a new fat-soluble vitamin that was called vitamin D.

Now that it was known that the antirachitic factor could be generated in the skin after exposure to sunlight or could be obtained from cod-liver oil, it became confusing as to whether there was more than one antirachitic factor. This issue was resolved.

FIG. 1. A typical scene in Glasgow in the mid-1800's as captured by this photograph taken by Thomas Annan. From Annan T (63), with permission.
when Powers et al. (10) reported that radiation from a mercury arc lamp had similar if not identical healing effects on rachitic rats when compared with those brought about by cod-liver oil. Once it was known that exposure to sunlight could prevent and cure the disease, Steenbock and Black (11) and Hess and Weinstock (12) independently demonstrated that exposure of food and a variety of other substances to the vitamin D producing radiation from a mercury arc lamp could impart antirachitic properties to these substances. This concept was first used to make milk antirachitic by adding the precursor of vitamin D to milk and then exposing the milk to radiation from a mercury arc lamp. Today vitamin D₂ or vitamin D₃ (Fig. 3) is directly added to milk [400 IU (10 µg) per liter] and other foods resulting in almost complete elimination of rickets in countries that use this practice.

CUTANEOUS PHOTOSYNTHESIS OF PREVITAMIN D₃

The sun emits a broad spectrum of radiation. The high energy photons that are most damaging to life on earth (below 290 nm) are absorbed by the thin layer of ozone that envelops the earth (3,13,14). When radiation between 290 and 315 nm
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FIG. 3. Structure of vitamins D₃ and D₂ and their respective precursors, 7-dehydrocholesterol and ergosterol. The only structural difference between vitamins D₂ and D₃ is their side chains: the side chain for vitamin D₂ contains a double bond between C₂₂ and C₂₄ methyl groups. From MacLaughlin JA and Holick MF (63).

(ultraviolet-B radiation) strikes the skin, approximately 10% is reflected and the other 90% is absorbed or scattered. The ultraviolet-B photons that are transmitted into the epidermis and dermis convert cytoplasmic stores of provitamin D₃ to previtamin D₃ (Fig. 4) (15). Once previtamin D₃ is made in the skin, it immediately begins to thermally equilibrate to vitamin D₃ by a temperature-dependent process (Fig. 4) (15). This thermal equilibration takes approximately 1½ to 2 days to reach completion at body temperature in humans. Because most of the previtamin D₃ synthesis occurs in the actively growing layers of the epidermis, which is in close proximity to the dermal capillary bed, changes in the temperature of the surface of the skin resulting from exposure to very warm or cold climates do not significantly alter the rate of conversion of previtamin D₃ to vitamin D₃ in warm-blooded animals. Although the exact mechanism for how vitamin D₃ exits the epidermal cells and is transported to the dermal capillary bed is unknown, there is evidence that the vitamin-D-binding protein which has a relatively high affinity for vitamin D₃ in comparison to previtamin D₃ and provitamin D₃ helps translocate vitamin D₃ from the epidermis into the dermal circulation (Fig. 4) (15).
FIG. 4. Schematic representation of the formation of previtamin D$_3$ in the skin during exposure to the sun and the thermal isomerization of previtamin D$_3$, which is specifically translocated by the vitamin-D-binding protein (DBP) into the circulation. During continual exposure to the sun, previtamin D$_3$ also photoisomerizes to lumisterol and tachysterol, which are biologically inert photoproducts (i.e., they do not stimulate intestinal calcium absorption). Because the DBP has no affinity for lumisterol and minimal affinity for tachysterol, the translocation of these photoisomers into the circulation is negligible, and these photoproducts are sloughed off during the natural turnover of the skin. Because these photoisomers are in a state of quasiphotoequilibrium as soon as previtamin D$_3$ stores are depleted (owing to thermal isomerization to D$_3$), exposure of lumisterol and tachysterol to ultraviolet radiation will provoke these isomers to photoisomerize to previtamin D$_3$. From Holick MF, et al. (17), with permission.

PHOTOCHEMICAL REGULATION OF CUTANEOUS VITAMIN D PRODUCTION

In 1967, Loomis (16) popularized a theory that skin pigmentation evolved for the purpose of regulating vitamin D$_3$ synthesis in the skin. He suggested that people living at or near the equator would have died of vitamin D intoxication as a result of daily exposure to intense solar radiation were it not for the evolution of more melanin pigmentation in the skin. Melanin is a natural sunscreen that is produced by the epidermis and can compete with provitamin D$_3$ for solar ultraviolet-B photons. As a result blacks and dark-skinned Asians require increased exposure to sunlight to produce the same amount of vitamin D when compared to Caucasians (3,17).

Although this was a plausible explanation for the evolution of melanin pigmentation it has been demonstrated that there is a more fundamental process that regulates the cutaneous production of vitamin D$_3$ in human skin. Previtamin D$_3$ is sen-
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FIG. 5. HPLC profiles of a lipid extract from the basal cells of surgically obtained hypopigmented skin that was previously shielded from (A) or exposed to (B–D) equatorial simulated solar ultraviolet radiation for 10 min (B), 1 h (C), or 3 h (D). E: Analysis of the photolysis of 7-dehydrocholesterol (7-DHC) in the basal cells and the appearance of the photoproducts previtamin D$_3$ (PreD$_3$), lumisterol (L), and tachysterol (T) with increasing time of exposure to equatorial simulated sunlight. From Holick MF, et al. (17), with permission.

sitive to both thermal energy and ultraviolet radiation. Once previtamin D$_3$ is formed in the epidermis and dermis it can either thermally isomerize to vitamin D$_3$ or during exposure to sunlight absorb ultraviolet-B radiation and isomerize to biologically inert isomers, lumisterol and tachysterol (Fig. 4) (17). Thus, during the initial exposure to sunlight provitamin D$_3$ is efficiently converted to previtamin D$_3$. During prolonged exposure to sunlight, however, there is little additional increase in previtamin D$_3$ production, because once formed previtamin D$_3$ is photoisomerized to biologically inert photoproducts (Fig. 5) (17).

Vitamin D$_3$ is also exquisitely sensitive to exposure to sunlight. As a result, any vitamin D$_3$ that is formed in the skin and does not escape into the circulation when exposed to sunlight is rapidly degraded into 5,6-trans-vitamin D$_3$, suprasterol I, and suprasterol II (Fig. 6) (2,17).

FACTORS THAT REGULATE THE CUTANEOUS PRODUCTION OF VITAMIN D$_3$

Photoproduction of previtamin D$_3$ in any layer of skin is dependent on the concentration of provitamin D$_3$, the presence of chromophores that compete with pro-
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FIG. 6. Photolysis of vitamin D$\textsubscript{3}$ to 5,6-transvitamin D$\textsubscript{3}$, suprasterol 1, and suprasterol 2. From Holick MF, et al. (60).

Vitamin D$\textsubscript{3}$ for ultraviolet-B photons, and the quantum of ultraviolet-B photons that are able to penetrate the skin and are absorbed by the provitamin D$\textsubscript{3}$ chromophore (3). The average concentration of provitamin D$\textsubscript{3}$ in a 6.2 cm$^2$ area of young adult human skin is approximately 5 $\mu$g for the epidermis and 1 to 3 $\mu$g for the dermis (18). Aging has a significant influence on the capacity of human skin to produce vitamin D$\textsubscript{3}$. There is an inverse relation between the concentration of provitamin D$\textsubscript{3}$ in the epidermis and age (Fig. 7) (18). Sunscreens which are effective in preventing the damaging effects of sunlight also prevent a beneficial effect of sunlight—the photosynthesis of previtamin D$\textsubscript{3}$ (19). The topical application of a sunscreen with a sun protection factor of only eight can completely block the photosynthesis of previtamin D$\textsubscript{3}$ in human skin and prevent the elevation in the concentration of vitamin D$\textsubscript{3}$ in the circulation after a whole body exposure to a dose of ultraviolet radiation that is equivalent to a minimal erythemal dose (Fig. 8) (19).

We have estimated that when a young adult is exposed to a whole body dose of sunlight that causes minimal erythema (1 minimal erythemal dose) the rise in the circulating concentration of vitamin D$\textsubscript{3}$ is comparable to the one resulting from an oral dose of 10,000 IU of vitamin D$\textsubscript{3}$. Therefore, the capacity of human skin to produce vitamin D$\textsubscript{3}$ is quite large and it has been recommended for the elderly in
FIG. 7. Effect of aging on 7-dehydrocholesterol concentrations in human epidermis and dermis. Concentrations of 7-dehydrocholesterol (provitamin D₃) per unit area of human epidermis (○), stratum basale (△), and dermis (◊) obtained from surgical specimens from donors of various ages. A linear regression analysis revealed slopes of -0.05, -0.06, and -0.0005 for the epidermis (r = -0.89), stratum basale (r = 0.92), and dermis (r = 0.04), respectively. The slopes of the epidermis and the stratum basale are significantly different from the slope of the dermis (P < 0.001). From MacLaughlin JA and Holick MF (18).

Boston, that 10 to 15 minutes of sunlight exposure of hands, face, and arms two to three times a week is more than adequate to provide the body with its vitamin D requirement (20).

The percentage conversion of cutaneous provitamin D₃ to previtamin D₃ is also very dependent on the solar zenith angle which is inversely related to the amount of ultraviolet-B photons in the solar spectrum. An increase in the zenith angle either by the daily rotation of the earth or by an increase in the distance north or south from the equator shifts the spectral distribution of sunlight towards longer wavelengths. As a result, less ultraviolet-B photons penetrate to the earth’s surface to promote cutaneous synthesis of vitamin D₃. It is well known that in northern latitudes children are more prone to developing rickets during and immediately after the winter when compared to the spring, summer, and fall. Originally it was thought that the principal cause for this was that children wore more clothing and were less outdoors. However, there is a more fundamental reason for the phenomenon. There is now evidence that exposure to sunlight in Boston (42°N) and Edmonton, Canada (52°N) does not result in any cutaneous production of previtamin D₃ between the months of November through February and October through March, respectively (Fig. 9).
FIG. 8. Serum vitamin D\textsubscript{3} concentrations (mean ± SEM) in eight normal subjects. Four subjects (○—○), had applied para-aminobenzoic acid (PABA) to the entire skin before exposure to ultraviolet-B. On day 0, all subjects underwent total-body exposure to one minimal erythema dose (1 MED) of ultraviolet radiation. Vitamin D\textsubscript{3} increased from day 0 to day 1 only in unprotected subjects (●—●) (p < 0.01). Changes in the PABA-treated group were not significant (p > 0.1). To convert nanograms of vitamin D per milliliter to nanomoles per liter, multiply by 2.58. From Matsuoka LY, et al. (64).

FIG. 9. [3α-\textsuperscript{3}H] 7-DHC in methanol was exposed to sunlight at different seasons and latitudes. The figure shows the mean ± 2 SD (n = 3) annual change in percent conversion of 7-DHC to previtamin D\textsubscript{3} after sunlight exposure for 1 hour (●—●) and 3 hours (○—○), and total photoproducts (previtamin D\textsubscript{3}, lumisterol, and tachysterol) after 3 hours (□—□) in Boston. The data were collected from November 1985 through 1986 to May 1987 and the figure shows compiled data for the 12 calendar months. For months where data was available for more than one year the results were the same within the uncertainty of a single point measurement, except for the photosynthesis of previtamin D\textsubscript{3} in May when exposure for 1 and 3 hours gave the same result in 1986 (●), and only a 1 hour value is available for 1987 (○). Also shown is the conversion of 7-DHC to previtamin D\textsubscript{3} throughout the year after exposure to 1 hour of sunlight in Edmonton (△—△), and in January in Los Angeles (LA) and Puerto Rico (PR). Single samples are accurate to ±1% photoproduct. From Webb AR, et al. (65).
In Buenos Aires which is approximately 35°S it is likely that the ineffectual time for vitamin D production is between the months of June through August. These observations provide a new insight into the role of sunlight and dietary or supplemental vitamin D in providing the body with its vitamin D requirement. It is probable that children and young adults make and store enough vitamin D during the spring, summer, and fall to get them through the winter months without developing vitamin D deficiency. However for the elderly and for very young children who may not be exposed to sunlight during the productive time of the year, their vitamin D stores may be low. Therefore during the winter months they would benefit from vitamin D supplementation in order to prevent vitamin D deficiency.

**METABOLISM OF VITAMIN D**

For most of the population of the world it is casual exposure to sunlight that is the principal source of vitamin D. The exceptions are probably people living in very northern and southern latitudes where the very oblique zenith angle of the sun does not permit sufficient vitamin D$_3$ production in the skin. Very few foods contain vitamin D naturally. Fish liver and visceral oils from some fish including cod and tuna fish and egg yolks contain vitamin D. Several countries practice the fortification of some foods with vitamin D. In the United States milk is the main dietary component subject to vitamin D fortification with either vitamin D$_2$ or vitamin D$_3$ (It should be noted that the structural difference between vitamin D$_2$ and D$_3$ resides in the side chain; vitamin D$_2$ contains a methyl group on carbon 24 and a double bond between carbons 22–23, vitamin D$_2$ and vitamin D$_3$ have equal biological activities in humans). Other countries fortify cereals, margarine, and breads with small quantities of vitamin D. The practice of fortification of foods with vitamin D has eradicated rickets as a significant health problem for these countries (3).

Once vitamin D$_3$ is synthesized in the skin or is ingested in the diet it enters the circulation and binds to a vitamin-D-binding protein. Vitamin D (the term without a subscript refers to either or both vitamin D$_2$ and vitamin D$_3$) is then transported to the liver where it is hydroxylated on carbon 25 to generate the major circulating form of vitamin D, 25-hydroxyvitamin D [25(OH)D] (Fig. 10) (22,23). The half-life of 25(OH)D in the human circulation is approximately 3 weeks and its concentration (normal ranges 8–55 ng/ml) is a good reflection of the accumulative effects of dietary intake and exposure to sunlight (23). The liver vitamin D-25-hydroxylase is relatively well regulated inasmuch as the relative increase in circulating concentrations of 25(OH)D in comparison to the cumulative intake of vitamin D$_3$ is relatively small (Fig. 11) (3,22,23). Patients with severe parenchymal and cholestatic liver disease often have low circulating concentrations of 25(OH)D (24). This is partly due to the associated intestinal malabsorption of vitamin D as well as a decrease in the reservoir of the vitamin D-25-hydroxylase in the liver. Institutionalized patients on multiple drugs for seizure control can often develop a 25(OH)D deficiency (3,25). The associated hypocalcemia, hypophosphatemia, and metabolic bone disease (rickets or
osteoalacia) can be corrected by increasing vitamin D intake. Patients with nephrotic syndrome also can have low circulating levels of 25(OH)D as a result of the loss of vitamin-D-binding protein which has 25(OH)D tightly bound to it (26). These patients will benefit also from increasing vitamin D intake from 100 IU to 4000 IU each day.

METABOLISM OF 25-OH-D TO 1,25(OH)₂D

25-Hydroxyvitamin D is biologically inert and requires a further hydroxylation in the kidney to 1,25-dihydroxyvitamin D (1,25(OH)₂D) (Fig. 10) (3,22,23,27,28). In most mammalian species including humans, the principal site for the metabolism of 25(OH)D to 1,25(OH)₂D is the kidney. However, during pregnancy the placenta plays a significant part in maintaining circulating concentrations of 1,25(OH)₂D by metabolizing 25(OH)D to 1,25(OH)₂D (29–31). The circulating half-life of 1,25(OH)₂D is approximately 4 to 6 hours and the normal circulating concentration (in our laboratory) is between 26 and 65 pg/ml. There have been reports that there are extrarenal sites for the production of this hormone, but all of these have occurred in cultured cells originating from bone, skin, or peripheral monocytes (3,32–34). It is known however, that patients who have chronic granulomatous disorders and a few patients with lymphoma have the capacity to metabolize 25(OH)D to 1,25(OH)₂D by a non-renal tissue (3,35–38). It is believed that this unregulated synthesis of 1,25(OH)₂D is responsible for the hypercalciuria and hypercalcemia associated with these dis-
Although the renal 25(OH)D-1α-hydroxylase is not sensitive to glucocorticoids, the non-renal enzyme that converts 25(OH)D to 1,25(OH)₂D is sensitive to steroid therapy. High dose prednisone has been effective in controlling the hypercalcemia and hypercalciuria associated with these disorders (37–39).

The renal 25(OH)D-1α-hydroxylase is found in the mitochondria and is believed to be regulated by a variety of ions and hormones (3,22,23,27,34). During periods of hypocalcemia there is a compensatory increase in parathyroid hormone secretion. Parathyroid hormone travels to the kidney and increases tubular reabsorption of calcium and the excretion of phosphate. In addition, possibly through its ability to decrease intracellular phosphate concentrations, this peptide hormone increases the conversion of 25(OH)D to 1,25(OH)₂D (Fig. 12). During periods of growth, pregnancy, and lactation, it is believed that growth hormone and prolactin indirectly upregulate the production of 1,25(OH)₂D (40–42). The increased production of 1,25(OH)₂D results in an increase in the efficiency of intestinal calcium absorption which satisfies the body's needs at these stressful times.

ALTERNATIVE METABOLISM OF 25(OH)D AND 1,25(OH)₂D

The body possesses a variety of enzymes that have the capacity to transform 25(OH)D and 1,25(OH)₂D into innumerable dihydroxy, trihydroxy, and tetrahydroxy metabolites (Fig. 13) (3,22,23,28,43,44). Most of the hydroxylations occur on the side chain and usually on carbons 23, 24, and 26. It is believed that these metabolic steps are related to its ultimate degradation to water-soluble compounds including calcitriol acid (3,22,23,43).

BIOLOGIC ACTIONS OF 1,25(OH)₂D

There is strong scientific evidence that 1,25(OH)₂D interacts with a specific high affinity binding receptor in the nucleus. This rare intracellular protein has a molecular weight in the range of 52,000 to 56,000 daltons (3,45,46). Once 1,25(OH)₂D₃ binds to its receptor, it activates this receptor and promotes binding of the receptor to acceptor sites (regulatory sequences) on nuclear DNA. It is believed that nuclear binding of the hormone–receptor complex regulates the transcription of hormone-specific mRNAs, which, in turn, governs the translation of proteins. One such protein has been identified as the calcium-binding protein (Fig. 14) (47).

1,25-Dihydroxyvitamin D₃ also maintains serum calcium levels by enhancing mobilization of calcium from bone. Originally it was believed that 1,25(OH)₂D₃ performed this function by interacting with osteoclastic receptors to increase osteoclastic activity. However, mature osteoclasts do not possess nuclear receptors for 1,25(OH)₂D₃ (48). It has been speculated that one of the physiologic functions of 1,25(OH)₂D₃ on bone is to mobilize stem cell mononuclear cells to become mature osteoclasts (Fig. 15) (3,45,49).
OTHER BIOLOGIC ACTIONS OF 1,25(OH)$_2$D$_3$

Recent evidence has suggested that a variety of other tissues in the body possess high affinity and low capacity nuclear receptors for 1,25(OH)$_2$D$_3$ (3,22,23,45,50). It has now been demonstrated that this hormone is extremely effective in inhibiting the proliferation of both normal and tumor derived cultured cells that possess its
**FIG. 13.** Pathway of 25(OH)D$_3$ metabolism to 25(OH)D$_3$-26,23-lactone. From Napoli JL, Horst RL (43).

**FIG. 14.** Proposed mechanism of action of 1,25(OH)$_2$D$_3$ in target cells resulting in a variety of biologic responses. CaBP, calcium-binding protein; 24-OHase, 25(OH)D-24-hydroxylase; Gla, osteocalcin; Alk. Pase, alkaline phosphatase; c-myc, c-fos, c-sis, proto-oncogenes. From Haussler MR, et al. (45).
FIG. 15. Proposed function of 1,25(OH)\(_2\)D\(_3\) and its receptor in bone remodeling and immunomodulation. From Haussler MR, et al. (45).

receptor (3,51). Although the physiologic importance of these \textit{in vitro} observations remains to be determined, \textit{in vivo} and \textit{in vitro} studies have revealed that 1,25(OH)\(_2\)D\(_3\) can inhibit the proliferation and induce cellular differentiation of 1,25(OH)\(_2\)D\(_3\) receptor positive cells and enhance or inhibit the synthesis and secretion of a variety of hormones, lymphokines, monokines, and immunoglobulins (3,52–55).

Initially it was thought that 1,25(OH)\(_2\)D\(_3\) would have great potential as an antitumor agent. However, it is now known that the antimitogenic activity of 1,25(OH)\(_2\)D\(_3\) is reversible and that tumor clones that have a decreased number of receptors for this hormone are resistant to its antimitogenic activity (56). Furthermore, when the hormone is given in pharmacologic doses it can cause severe hypercalcemia (57). Despite these problems, 1,25(OH)\(_2\)D\(_3\) may be of great pharmacologic value, especially for dermatology. Unlike tumor cells that can dedifferentiate when 1,25(OH)\(_2\)D\(_3\) is removed from their environment, when 1,25(OH)\(_2\)D\(_3\) induces human keratinocytes to differentiate, it does so in an irreversible manner. One practical application is the effective use of 1,25(OH)\(_2\)D\(_3\) for the treatment of the hyperproliferative skin disorder psoriasis (2,58–60).

**NON-NUCLEAR MEDIATED EFFECTS OF 1,25(OH)\(_2\)D\(_3\)**

Although it is well documented that most of the biologic actions of 1,25(OH)\(_2\)D\(_3\) occurred through its interaction with a specific nuclear receptor, there is mounting evidence that in some cells 1,25(OH)\(_2\)D may also increase intracellular calcium con-
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centrations and alter phosphoinositol metabolism in both receptor positive and negative cells (2,61). The physiologic role of these "rapid" actions of 1,25(OH)2D remains to be determined.

CONCLUSION

It is remarkable that exposure to sunlight should play such a significant role in maintaining calcium homeostasis and bone metabolism in humans. Although it is difficult to understand how sunlight became involved in such vital processes, it is well recognized that vitamin D is absolutely essential for the health and development of the growing skeleton. It is not often appreciated, however, that vitamin D is also required for the proper remodeling and maintenance of bone throughout our entire lives. Furthermore, it is casual exposure to sunlight during daily activities that is responsible for providing most of our vitamin D requirement.

Sunlight not only provides the energy to ultimately produce vitamin D3 in the skin, but it is also responsible for regulating the total amount that can be produced thus preventing vitamin D intoxication during excessive exposure to sunlight. Aging, sunscreens, changes in the zenith angle of the sun, and skin pigmentation all can limit the cutaneous production of vitamin D3. Once formed, vitamin D3 is a biologically inert substance that is metabolized first in the liver and then in the kidney to its biologically active form, 1,25(OH)2D. This hormonal form of vitamin D is responsible for increasing the efficiency of the intestine to absorb dietary calcium, and also to mobilize calcium from bone at times when dietary calcium is unable to meet the body's requirement for calcium. During the past decade, new revelations about 1,25(OH)2D3 have suggested that this hormone may have a variety of other biologic actions in the body. *In vivo* and *in vitro* studies have suggested that 1,25(OH)2D3 is a potent antiproliferative agent that is also capable of inducing differentiation. In addition, 1,25(OH)2D3 has been shown to have immunomodulatory effects as well as influencing the secretion of a variety of hormones, and lymphokines. At first glance it would appear that 1,25(OH)2D3 is absolutely essential for the function of many of these physiologic actions. However, the reader should note that patients with vitamin D-dependent rickets type II (these patients often have a defective or absent receptor for 1,25(OH)2D3) (see chapter by S. Marx) who are unresponsive to 1,25(OH)2D3 and have all of the manifestations of vitamin D deficiency including hypocalcemia, hypophosphatemia, and rickets do not show any significant abnormalities in their immune function, hormonal secretory reserve, nor do they have an increased incidence of hyperproliferative disorders. The fact that 1,25(OH)2D3 is not essential for controlling cell proliferation and differentiation in tissues that possess its receptor does not diminish its potential as a new pharmacologic agent for treating a variety of hyperproliferative disorders such as leukemia and psoriasis. Although at the present time 1,25(OH)2D3 has not proven useful for leukemia, it has been effective in the treatment of some patients with psoriasis.

The vitamin D story continues to evolve. The recent evidence that 1,25(OH)2D3
may alter intracellular calcium movement and affect phosphoinositol metabolism offers a major new insight into the potential physiologic and pharmacologic action of 1,25(OH)₂D₃. These observations coupled with previous observations regarding this hormone's antiproliferative activity should herald a new dimension in the understanding of the physiologic action of this most interesting vitamin/hormone.

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DISCUSSION

Dr. Pettifor: In tropical or subtropical environments, is it possible Caucasians although producing more pre-vitamin D3 and thus vitamin D3, will have most of it destroyed with continued sun exposure, while blacks will produce less pre-vitamin D3 but also have a lesser inactivation of the skin produced vitamin D3?

Dr. Holick: That is correct.

Dr. Guesry: Do you have any insight into the possible role of vitamin D in muscle weakness? Is it related to phosphorus or is it a direct effect of the vitamin D metabolites?

Dr. Holick: At least in culture, skeletal muscle cells possess receptors for 1,25(OH)2D3.
and respond to it. It may be that the metabolite can mobilize intracellular calcium pools to help in skeletal muscle function. Would Dr. Boland care to comment about this?

Dr. Boland: Indeed in differentiated skeletal muscle, \(1,25(OH)\_2D\) seems to play a role in the regulation of intracellular calcium. There is evidence, using animal models, that \(1,25(OH)\_2D_3\) affects the activity of the calcium pump located in the sarcolemma and possibly the concentration of the calcium ATPase in the sarcoplasmic reticulum.

Dr. Glorieux: There is clinical evidence for the importance of vitamin D, and not of phosphorus, in muscle strength. One of the major differences between familial hypophosphatemic rickets (HYP) on one hand, and D-deficient or D-dependent rickets on the other is muscle weakness. All patients are hypophosphatemic, but HYP subjects, who have normal \(1,25(OH)\_2D\) serum levels do not have muscle weakness, while in the other cases \(1,25(OH)\_2D\) levels are low and proximal myopathy is evident. In the latter patients treatment with \(1,25(OH)\_2D\) induces rapid recovery of muscle strength before correction of hypophosphatemia.

Dr. Marx: You and others have shown vitamin D synthesis in single cell organisms, such as phytoplankton. We do not yet recognize a role for vitamin D in these organisms. Can you indicate when and why, during evolution of species, \(1,25(OH)\_2D\) assumed an important role in extracellular calcium metabolism?

Dr. Holick: Because the ocean environment is high in calcium and relatively low in phosphorus, it is likely that vitamin D began to play a significant role in calcium metabolism at the time when vertebrates began to crawl on the Earth's surface. At that time there was a great need to increase the utilization of the minute quantities of calcium that was available.

I have speculated that provitamin D and vitamin D may have evolved to (a) act as a natural sunscreen, (b) function as a photochemical signal, and (c) change membrane fluidity. Once provitamin D is photolyzed to previtamin D the rigid provitamin D structure becomes more flexible thus changing its physical and chemical properties. The same is true for previtamin D's thermal product vitamin D\(_3\). The carbons that would be most prone to autohydroxylation are carbons 1 and 25. It is likely in evolution that the addition of hydroxyls on carbons 1 and 25 made the molecule more hydrophilic and as a result enhanced its ability to alter membrane permeability to ions such as calcium. During evolution this property offered an advantage and eventually enzymes evolved to make the seco-steroid, \(1,25\)-dihydroxyvitamin D.

Dr. Boland: You said that the effect of parathormone on the synthesis of \(1,25(OH)\_2D\) would be mediated by a decrease in intracellular phosphate concentration in the kidney cell. Could you further elaborate on that?

Dr. Holick: Rat studies have shown that in the absence of parathyroid hormone low blood phosphorus and not low blood calcium stimulated the production of \(1,25(OH)\_2D\).

Dr. Markestad: You suggested that sunlight exposure will be less efficient for epidermal synthesis of vitamin \(D_3\) the further you get away from the equator since less UV-B photons will penetrate to the earth. In Norway, which covers the latitudes of approximately 58–72\(^\circ\) North, we observed no significant regional differences in \(25(OH)D\) levels during summer, and it appears that very little sunshine exposure is needed to produce adequate \(25(OH)D\) concentrations. Scandinavians probably benefit from their fair skin.

Dr. Holick: I agree. In summer time the zenith angle of the sun is less oblique which would permit the efficient production of previtamin D in the skin. At other times of the year dietary fatty fish serve as an excellent source of vitamin D.

Dr. Elidrissy: We have observed that, in all parts of Saudi Arabia, levels of vitamin D are lower than those in Western countries. We also did not find a difference between dark skin
pigmentation and the general Arab complexion. Do you think that other factors may limit the increase in vitamin D levels in very sunny countries? Are these low levels normal for such countries?

Dr. Holick: In countries like sunny Saudi Arabia, minimal exposure to sunlight will induce the synthesis of previtamin D$_3$. Continued exposure however will result in the photodegradation of previtamin D$_3$ to lumisterol. Therefore production of previtamin D$_3$ cannot be greatly increased. The same holds true for vitamin D$_3$. Vitamin D$_3$ is very sensitive to photodegradation by the sun. So, in countries such as Saudi Arabia where the sun shines almost everyday of the year, 25-hydroxyvitamin D levels should not be different in light and dark skin people. There is another potential explanation based on studies we have conducted in reptiles that I did not have time to mention. When exposed to sunlight amphibians and reptiles produce at least six different previtamin Ds in their skins. We found that there are at least two provitamin Ds in mammalian skin. One of these provitamin Ds, 24-dehydroprovitamin D$_3$, is converted to 24-dehydroprovitamin D$_3$, which is thermally isomerized in the skin to 24-dehydrovitamin D$_3$. When 24-dehydroxyvitamin D$_3$ reaches the liver, it can inhibit the conversion of vitamin D$_3$ to 25-hydroxyvitamin D$_3$. Thus, it seems that this vitamin D can influence the liver’s capacity to produce 25(OH)D. During excessive exposure to sunlight, this mechanism may be operative. Another explanation is that the men and women cover most of their skin surface with clothing which also reduces vitamin D synthesis.

Dr. Boland: There are conflicting reports concerning the effects of 1,25(OH)$_2$D on cell proliferation. You mentioned that the hormone suppressed keratinocyte proliferation and there are reports showing that 1,25(OH)$_2$D$_3$ stimulates cell proliferation. We have seen in muscle cells, low 1,25(OH)$_2$D$_3$ levels (10$^{-10}$-10$^{-11}$M), stimulate cell proliferation. What is the relationship between those two effects? Can you make some connection with the mechanisms of action of the hormone, specifically the genomic mechanism, or perhaps an effect at the membrane level that you related to the rapid action of 1,25(OH)$_2$D$_3$ in skin cells?

Dr. Holick: In skin cells we have shown that at 10$^{-12}$ M 1,25(OH)$_2$D increased proliferative activity. However, at pharmacological levels, the hormone inhibited proliferation. We do not yet understand the mechanism for this. Some have suggested that if you look at c-myc oncogen expression as one of the markers for proliferation, it is inhibited by 1,25(OH)$_2$D. When you take the hormone away, the gene is once again expressed, suggesting that there may be some direct relationship. It also is quite conceivable, that 1,25(OH)$_2$D increases phosphoinositol metabolism, and intracellular calcium pools which in turn increase or decreases cell proliferation.

Dr. Boland: Eventually the rapid changes in intracellular calcium concentration might affect oncogene expression.

Dr. Holick: Yes.

Dr. Arnaud: Dr. Bikle from our Unit, has demonstrated that keratinocytes can make 1,25(OH)$_2$D. The cells do not release in into the medium which would suggest that if it is of physiological importance, 1,25(OH)$_2$D is produced as an autocrine factor. Do you know whether psoriatic cells are capable of making 1,25(OH)$_2$D and whether or not that might be the defect that ultimately leads to the development of psoriasis.

Dr. Holick: Because it is very difficult to grow psoriatic keratinocytes, we have not yet been able to answer your question. In fact, we do not know whether what Dr. Bikle has shown very nicely *in vitro* occurs *in vivo*. We have not been able to demonstrate the synthesis of 1,25(OH)$_2$D$_3$ in the skin of nephrectomized rats.