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REVIEW

Vitamin D in Nature: A Product of Synthesis and/or Degradation of Cell Membrane Components

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Abstract—The review discusses the data on vitamin D accumulation in animals, plants, and other organisms. 7-Dehydrocholesterol (7-DHC) and ergosterol are considered to be the only true precursors of vitamin D, although even vitamin D_2 (ergocalciferol) is not fully comparable to vitamin D_3 (cholecalciferol) in regard to their functions. These precursors are converted by UV radiation into the corresponding D vitamins. There are a few published reports that this reaction can also occur in the dark or under blue light, which is unexpected and requires explanation. Another unexpected result is conversion of pro-vitamins D (7-DHC and ergosterol) into vitamin D_3 and D_2 via pre-vitamin D at low temperatures (<16°C) in the lichen *Cladonia rangiferina*. An extensive survey of literature data leads to the conclusion that vitamin D is synthesized from (1) 7-DHC via lanosterol (D_3) in land animals; (2) 7-DHC via cycloartenol (D_3) in plants; (3) ergosterol via lanosterol (D_2) in fungi; and (4) 7-DHC or ergosterol (D_3 or D_2) in algae. Vitamin D primarily accumulates in organisms, in which it acts as a pro-hormone, e.g., land animals. It can also be found as a degradation product in many other species, in which spontaneous conversion of some membrane sterols upon UV irradiation leads to the formation of vitamins D_3 or D_2, even if they are not necessarily needed by the organism. Such products accumulate due to the absence of metabolizing enzymes, e.g., in algae, fungi, or lichens. Other organisms (e.g., zooplankton and fish) receive vitamins D with food; in this case, vitamins D do not seem to carry out biological functions; they are not metabolized but stored in cells. A few exceptions were found: the rainbow trout and at least four plant species that accumulate active hormone calcitriol (but not vitamin D) in relatively high amounts. As a result, these plants are very toxic for grazing animals (cause enzootic calcinosis). In connection with the proposal of some scientists to produce large quantities of vitamin D with the help of plants, the accumulation of calcitriol in some plants is discussed.

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At present, the importance of vitamin D should be considered a global issue [1, 2]. The functions of vitamin D extend far beyond its participation in the bone tissue metabolism [3]. The vast majority of people and animals receive only a small amount of vitamin D (~10% or even less) with food [3]. In humans, the main source of vitamin D_3 is its synthesis in skin from the precursor 7-dehydrocholesterol (7-DHC) upon exposure to UV radiation. Unlike cholecalciferol (vitamin D_3), humans and animals receive ergocalciferol (vitamin D_2) exclusively from food. Therefore, *ergocalciferol, in contrast to cholecalciferol, is a true vitamin for them.*

A scheme that is widely used when discussing vitamin D synthesis was suggested by Holick et al. [4]. This scheme was primarily developed for vitamin D synthesis in humans. Further in this paper, we will discuss the problem of vitamin D formation in a broader sense, since new data have recently appeared in the published literature that should be taken into consideration. In particular, it was found that vitamin D-dependent organisms can exist under a wide variety of conditions, for example, at ambient temperatures below 15-16°C and at very low intensity of UV radiation [5, 6].

The stages of calcitriol synthesis are shown in the scheme. Let us consider in more detail the part of this scheme that preceeds vitamin D_3 formation.

VITAMIN D PRECURSORS

It is generally accepted that 7-DHC and ergosterol are two initial compounds in the chain of reactions of

Abbreviations: 7-DHC, 7-dehydrocholesterol; UV, ultraviolet; vitamin D_2, ergocalciferol; vitamin D_3, cholecalciferol.

1) 7-DHC (provitamin D) $\xrightarrow{h\nu}$ **"previtamin D"**

(Are there other precursors? Is UV radiation necessary?)

2) "previtamin D" $\xrightarrow{+25\,°C}$ **vitamin D_3**

(At what temperature can thermal isomerization take place?
Is vitamin D_3 inactive intermediate?)

3) vitamin D_3 $\longrightarrow$ **$25(OH)D_3$ (calcidiol)**

(Inactive circulating form?)

4) $25(OH)D_3$ $\longrightarrow$ **$1,25(OH)_2D_3$ (calcitriol)**

(Hormone active form)

Scheme of calcitriol synthesis (based on the study by Chen et al. [4] with modifications)

vitamin D synthesis. However, if we discuss this issue in more general terms, i.e., search for the precursors of compounds that possess the properties or functions of vitamin D, the range of such precursors becomes much wider.

Firstly, there is a group of other sterols that could be converted into substances similar to vitamin D in the UV-induced photochemical reaction [7]:

22,23-dehydroergosterol → vitamin D_4;
7-dehydrositosterol → vitamin D_5;
7-dehydrostigmasterol → vitamin D_6;
7-dehydrocampesterol → vitamin D_7.

As early as 1968, the first study was published that compared distribution and functions of vitamins D_4 and D_3 in rat tissues [8]. Unfortunately, no results were obtained that would have allowed the representatives of this group to be considered functionally similar to vitamins D_3 or D_2. Therefore, there is no reason to consider the original sterols as vitamin D precursors as well.

Secondly, 7-DHC is hydroxylated by CYP11A1 with the formation of 22(OH)-7-DHC and 20,22-$(OH)_2$-7-DHC that are further converted into 7-dehydropregnenolon and, finally, into Δ7-steroids. In skin, Δ7-steroids form secosteroids under UV radiation. Their physiological activity is similar to the activity of some vitamin D metabolites. For example, 21(OH)-pregnacalciferol inhibits formation of melanoma cell colonies to the same extent as $1,25(OH)_2D_3$ [9]. At the same time, these compounds display no typical hormonal effects on bone metabolism and other organism functions.

Assuming that we are searching for a precursor in the synthesis of active calcitriol hormone or a functionally similar compound, *we can reliably refer only to 7-DHC and ergosterol.*

VITAMIN D AND UV RADIATION

In our earlier review, we concluded that "without the UV part of the solar radiation spectrum, no vitamin D would be present on the Earth at all!" [3]. This statement is based on the data of many papers published already in the first years of studying vitamin D [10-13]. It is assumed that the B ring in the 7-DHC molecule can be opened by the energy of photons (18 mJ/cm^2) in the 282-310 nm range, which corresponds to UVB radiation [14, 15].

However, the results of some studies contradict this view. A new mechanism of vitamin D synthesis in the absence of UV radiation was discovered in cultured *Solanum glaucophyllum* cells [16, 17]. In these cells (both in tissue culture and in suspension), the B ring in 7-DHC was opened in the dark. Unfortunately, the cited studies did not provide explanation for the observed phenomenon.

Several years ago, the effect of UVB radiation on the accumulation of vitamin D and its hydroxylated metabolites in plants from the Solanaceae family was studied in special climatic chambers [18]. In these experiments, control plants were grown at a 16 h light/8 h dark cycle without UV irradiation. If even a small fraction of UV were present in the light source, photochemical formation of certain amounts of vitamin D and 25(OH)D should have been expected. And if not? In the review [19] published soon after, the authors wrote that "vitamin D_3 synthesis without the action of UVB has also been reported".

Another interesting example is formation of vitamin D from 7-DHC in the skin of rainbow trout (*Oncorhynchus mykiss*) under *blue light* (380-480 nm). It is interesting that in this case as well, $1,25(OH)_2D_3$ was accumulated instead of vitamin D_3 or $25(OH)D_3$. It should also be noted that in trout, calcitriol is synthesized in the liver and not in kidneys (as in land animals) [20].

Nevertheless, considering a large number of published works and theoretical considerations, one should proceed from the assumption that UV radiation is essential for the photochemical reaction of 7-DHC and ergosterol conversion. Under its action, photolysis of 7-DHC and ergosterol can occur even in a test tube with hexane [21] or other solvents of lipids. This reaction proceeds faster by an order of magnitude when the sterols are embedded in biological membranes. It also takes place under the sunlight in biological waste (e.g., in feces) or, for example, in hay [3]. At the same time, the rate of this process largely depends on already existing conditions (see below).

VITAMIN D FORMATION AND CONDITIONS FOR THERMAL ISOMERIZATION

The optimal condition for the pre-vitamin D thermal isomerization is considered to be at a temperature of ≥25°C [13, 21]. Such temperature conditions may fully explain the wide occurrence of vitamin D in nature: in the skin of warm-blooded (homoiothermic) animals, on land, and in the upper layers of the oceans, reservoirs and rivers in equatorial areas inhabited by vast amounts of plankton organisms of various phylogenetic origin. The membranes of plankton cells contain many different sterols, including 7-DHC and ergosterol.

However, humans and animals also live in colder territories away from the coasts, for example, in the Arctic and Subarctic tundra. Although the intensity of UV radiation in the far-north regions is low, it remains sufficient for the synthesis of vitamin D from sterols [22, 23], especially in summer, when the sun shines for almost 24 h a day.

Another important question is the role of thermal isomerization in the process of vitamin D formation from the pre-vitamin in arctic conditions. According to the scheme, a temperature of ≥25°C is required for pre-vitamin D thermal isomerization [13]. However, in this case, specific conditions of isomerization are important [4]. If conversion of 50% previtamin D into vitamin D at 37°C in hexane solution takes 30 h, achieving the same result in human skin at the same temperature takes only 2.5 h [24, 25]. Not only the equilibrium of this reaction is shifted toward the vitamin D formation, but the reaction rate is increased by more than 10 times [25]. The reaction rate changes significantly in aqueous medium in the presence of β-cyclodextrin [26]. Under these conditions, the reaction rate constant for previtamin D conversion into vitamin D, as well as the reaction rate constant for the reverse reaction, increases more than 40 and 600 times, respectively, compared to the reaction in *n*-hexane.

It is known that vitamins D_2 and D_3 are accumulated, for example, in the reindeer lichen *Cladonia rangiferina* in the Arctic and Subarctic regions [5, 27], where the maximum temperature rarely exceeds 16°C (mostly stays within 5-10°C in summer). Unfortunately, there are little data on the rate of vitamin D formation under these conditions, when all biological processes occur at extremely slow rates. The annual growth rate of reindeer lichen is ~15 mm at its best. The vitamin D content in the lichen depends not only on the rate of its synthesis, but also on the rate of its metabolism (see the next section).

In some organisms, thermal isomerization of previtamin D into vitamin D occurs at temperatures significantly below 25°C. Do these organisms possess specific adaptations or "catalysts" for the thermal isomerization of previtamin D into vitamin D at low temperatures? Might vitamin D accumulation occur due to its low-rate formation in the absence of metabolization or at its significant slowdown?

WHICH ORGANISMS SYNTHESIZE VITAMIN D PRECURSOR?

It is obvious that the formation of the precursor is a prerequisite for vitamin D synthesis, since the next stage of this process is carried out without any contribution from specific enzymes. Certainly, one can expect that morphological and anatomical features, as well as intracellular compartmentalization of the reaction components, may influence the rate of formation and the quantity of the reaction products. Plants synthesize a large variety of chemical compounds, but this does not mean that these compounds may be found in tissues in more or less significant amounts, since they may be intermediate products in the chains of reactions. On the other hand, if a compound is consumed with food by an organism lacking enzymes for further metabolization of this compound, it simply accumulates in the cells. Fat-soluble products accumulate in special fat cells (adipocytes), as vitamin D does in animals [28-30].

To illustrate this, let us consider an organism that is often mentioned in literature on vitamin D, namely the planktonic coccolithophore *Emiliania huxleyi*. This species of plankton has existed in the Sargasso Sea for over 750 million years [11, 31]. In terms of dry weight, *E. huxleyi* contains up to 0.1% ergosterol. Ergosterol is an essential component of cell membranes of many organisms. If plankton accidentally enters the upper layers of the ocean, a certain portion of ergosterol is converted into vitamin D_2 by UV radiation at sufficiently high temperatures. This process occurs without any participation of specific enzymes. The product of this accidental exposure to UV radiation does not undergo further metabolization, since cells do not have the necessary enzymes. *In this case, vitamin D_2 is most likely a product of damage or even degradation of biological membranes under the action of UV radiation.*

Which organisms are capable of vitamin D synthesis and which organisms accumulate it as a degradation product? *7-DHC and animals.* The fact that in the animal world, 7-DHC serves as a precursor for the synthesis of vitamin D under the action of UV light is generally accepted. This process takes place in most land animals, from amphibians to primates [11]. In animals, cholesterol is formed from lanosterol [32, 33]. At the same time, 7-DHC and cholesterol are also found in many plants, algae, and cyanobacteria.

7-DHC and plants. For a long time, it had been assumed that only vitamin D_2 is synthesized in plants. The synthesis of vitamin D_3 was discovered later [34]. In fact, all data demonstrating vitamin D_2 formation in plants result from contamination of plant material with fungal hyphae [35-37]. Although plants possess a fairly high number of sterols, they lack ergosterol and, consequently, vitamin D_2 [38]. In plants, 7-DHC is commonly found in very low concentrations, amounting to only 1-2% of the total content of sterols, although in some plants, the concentration of 7-DHC can be approximately an order of magnitude higher [39-44].

Vitamin D_3 and its metabolites, including calcitriol ($1,25(OH)_2D$), were found in some plants. The following plants contain vitamin D_3 and its metabolites, including calcitriol, in relatively large amounts: *Solanum malacoxylon* [45], *Lycopersicon esculentum* [46, 47], *S. tuberosum* [33, 46], *S. melongena* [46], *Cucurbita pepo* subsp. *pepo* convar. *giromontina* [46], *S. glaucophyllum* (plants and cell culture) [16, 17, 48], *Nicotiana glauca* [33, 49], *Cestrum diurnum* [50], and *Trisetum flavescens* [51, 52]. It is surprising that some of these plants not only synthesize vitamin D_3, but also accumulate extremely high concentrations of calcitriol. This primarily applies to four species: *S. malacoxylon* [45], *S. glaucophyllum* [48], *C. diurnum* [53], and *T. flavescens* [51, 52] that prove to be very dangerous for livestock (cows, sheep, horses, goats, pigs, and other animals). After eating them, animals fall ill with hypercalcemia (enzootic calcinosis). This disease develops as a result of massive intake of vitamin D_3, as well as its active metabolite calcitriol. In Europe, a common cause of hypercalcemia is the yellow oat grass *T. flavescens* that typically grows in the European Alps and the Caucasus [51, 52, 54, 55]. The toxic effect of this plant is greatly increased after it dries in the sun [56]. In South America, the toxic effects of vitamin D in some animals can result from consumption of *S. glaucophyllum* and *S. malacoxylon* of the Solanaceae family. *Cestrum diurnum*, which is another Solanaceae species often consumed by livestock, grows in North America (USA) [50].

It had been believed before that plants do not contain vitamin D_3. This view was based, on one hand, on a very low vitamin D_3 content in most plants [32], and on the other hand, the use of insufficiently sensitive analytical methods [19]. In recent years, some authors have come to the conclusion that cholesterol is characteristic of all plants, since it is an intermediate product in the synthesis of steroid glycoalkaloids and phytoecdysteroids [32]. Cycloartenol is considered to be a precursor of 7-DHC in plants [32, 33].

7-DHC and algae. Cholesterol was found in green [57], brown [58] and red [59] algae [19]. Seckbach and Ikan [59] suggested that cholesterol apparently occurs in all groups of plants, since it occupies a key position in the biosynthesis of other steroids.

Ergosterol and fungi. The presence of ergosterol, often in relatively high concentrations, is characteristic of all fungi [60]. Ergosterol is a dominant sterol in fruit bodies of wild and cultivated fungal species [61]. Moreover, three other sterols were found in fungi, although in low concentrations: ergosta-7,22-dienol, ergosta-5,7-dienol, and ergosta-7-enol. The content of vitamin D_2 in fungal fruit bodies could be increased 9- to 14-fold by UV irradiation at 254 nm for 2 h at a distance of ~20 cm. Similar data were obtained in other laboratories as well [62, 63]. These results demonstrate that fungi possess significant reserves of ergosterol that could be converted into vitamin D_2 by UV radiation. Ergosterol in fungi, like 7-DHC in animals, is synthesized from lanosterol [32].

Ergosterol and algae. Ergosterol was also found in various species of algae, e.g., *Dunaliella tertiolecta* [64], *Chlamydomonas reinhardtii* [65, 66], *Chlorella vulgaris* [67], and *Cyanidium caldarium* [59, 68] (see also the study by Bjorn and Wang [6]).

Therefore, the pathways for the synthesis of vitamin D precursors vary in different groups of organisms:

– land animals – 7-DHC from lanosterol;

– higher plants – 7-DHC from cycloartenol;

– fungi – ergosterol from lanosterol;

– algae – 7-DHC or ergosterol.

WHICH ORGANISMS ACCUMULATE VITAMIN D?

Up to this moment, we have been mainly interested in the occurrence of vitamin D and its precursors in various organisms and only briefly touched the issue of its accumulation. After all, not all animals require vitamin D; nevertheless, many of them can accumulate it in significant amounts.

For example, plankton, which is the first link in the food chains for many animals, is rich in vitamins D_2 and D_3. This is particularly typical of plankton in tropical and subtropical regions. Water circulation in the oceans, in particular the Gulf Stream current, carries plankton to distant oceans and seas. In this way, vitamin D is transported, for example, far north, where it performs an important function in land animals and humans [3]. However, this benefits only coastal regions, as well as regions permanently covered with ice.

There are small nation populations inhabiting, for example, the northern regions of Russia (between latitudes of 57-67°N) [69]. They live relatively far from sea coasts and are almost completely deprived of vitamin D sources from the sea plankton. UV radiation in far-northern habitats is clearly insufficient for providing humans (and animals?) with required amounts of vitamin D. Their survival depends on the presence of vitamin D in food. In this case, the initial source of vitamin D is lichens. Ergosterol and 7-DHC, as well as vitamins D_2 and D_3, were found in the reindeer lichen (*Cladonia rangiferina*) that grows in the far north of Russia and other countries (57-70°N) [27, 69]. This symbiotic organism consisting of a fungus and an alga accumulates vitamins D_2 and D_3. It forms the first link in a food chain, since it is the main food source for reindeer in winter. Reindeer accumulate D vitamins in muscle tissue, giving humans and predatory animals an opportunity to receive vitamin D in sufficient quantities.

The concentrations of vitamins D_3 and D_2 in the reindeer lichen are within 67-204 and 22-55 μg per 100 g of dry matter, respectively [70]. Moreover, reindeer lichen also has high ergosterol content. For example, let us compare the known numbers for certain food products with a high vitamin D concentration (μg per 100 g of *fresh* matter): eggs – 0.4-12; cheese – 1.25; chicken liver – 1.20; beef liver – 0-14.1; herring – 2.2-38.0; trout – 4.2-34.5; fungi – 0.3-30.0. Reindeer lichen exceeds all the above-mentioned products in its vitamin D content.

A question arises on how can lichens form vitamin D under these harsh conditions? Calculations demonstrate that even at 70°N, the daily dose of UV radiation in summer may be quite sufficient for vitamin D biosynthesis in lichens [23]. It should be emphasized that even if the growth rate of reindeer lichen is very low (~3-20 mm per year), it grows on vast areas of tundra and light forests of taiga. In winter, reindeer lichen is the main food source for reindeer, which consume only the uppermost part of lichen branches. In addition to reindeer, foxes, arctic foxes, snow sheep, wolves, lemmings, brown hares, and some other animals can be found in northern regions. These regions are sparsely inhabited, but people began to populate them already ~45,000 years ago [71], when they moved from North-East Siberia to the west and occupied these places as nomadic hunters or reindeer shepherds.

There are many organisms in which the concentrations of vitamin D and its precursors are very low. Plants are a good example. They possess these compounds only as intermediates. In other cases, vitamin D can be accumulated in significant amounts, for example, in various algae that use sterols as building materials for the membranes. Such sterols, including UV-sensitive cholesterol and ergosterol, are converted into vitamins D_2 and D_3 under high-intensity UV radiation at relatively high temperatures of water. The mechanism of formation of these vitamins is clear; however, it is still unknown whether they possess any function in these organisms. Most likely, they do not. From an evolutionary viewpoint, as long as accumulated vitamin D does not harm the cells in which it is formed, its excessive accumulation does not matter for the natural selection. It may be assumed that similar passive accumulation occurs in representatives from the protozoa to aquatic lower vertebrates, as well as in fungi and lichens.

The situation is quite different in land animals, including humans. These organisms can accumulate additional amounts of vitamin D as a result of its synthesis under UV radiation or consumption with food. In this case, a portion of vitamin D is accumulated as a reserve together with other fat-soluble compounds (e.g., vitamins A, E, and K) in adipocytes.

However, some cases were described that do not fit into this scheme. For example, the blood levels of vitamin D in naked mole rats living in the underground colonies are very low, but at the same time, these animals have a high content of the Klotho protein, a component of the anti-aging mechanism, whose role in life in the dark is currently being studied [72]. In his review [72], Dammann also discusses alterations in the Klotho levels in another group of nocturnal animals, bats.

"IN PURSUIT OF VITAMIN D IN PLANTS"

Articles with similar titles have appeared in scientific journals, in which the authors declare the urge for intensifying efforts for creating plant mutants capable of vitamin D synthesis and accumulation [19, 38, 73]. Some researchers believe that plants can also synthesize vitamin D in the dark [73] (see the section "Vitamin D and UV radiation"); for this reason, they call for studying the capacity of underground plant organs to synthesize vitamin D. Other scientists refer to the necessity for UV radiation in this synthesis and emphasize that vitamin D is synthesized in animal skin and plant *leaves* [38].

The idea of using plants as a source of organic vitamin D to meet human needs is tempting. Indeed, plants with high vitamin D content have been found (four species so far; see section "7-DHC and plants"). Unfortunately, these plants contain not only vitamin D_3, but even higher concentrations of active calcitriol. While unicellular organisms accumulate vitamin D as the final product and land animals accumulate it in the form of $25(OH)D_3$, in these four plant species, synthesized vitamin D is immediately hydroxylated, forming $25(OH)D_3$, which is then converted into calcitriol.

The corresponding hydroxylases in plants are so active that the entire chain of reactions of calcitriol formation occurs without any kind of reverse regulation. The accumulation of significant amounts of calcitriol indicates the absence of its metabolization in plant tissues. And how can the reaction chain be stopped at the vitamin D stage? Cultivated plants with high calcitriol content would be poisonous and unfit for human or animal consumption (see section "7-DHC and plants").

Using vitamin D from plant sources is likely to be complicated. *Is it possible that using algal cultures would be a more promising approach to producing vitamin D-enriched food products?*

If we proceed from the scheme, the last two reactions remain out of our scope. They are rather linked to the issue of *the mechanism of vitamin D action*. Neither the role of vitamin D_2, nor that of other D vitamins and their analogs are discussed in this review. If we consider the functions of vitamin D in treating rickets and some other bone diseases, then D_2 should be obviously placed in the group of D vitamins [62]. In literature, vitamins D_3 and D_2 are often compared with respect to the accumulation of the corresponding forms of 25(OH)D [74-76]. Some authors believe that vitamins D_2 and D_3 are equally available biologically, regardless of whether they are consumed with food or taken in capsules [77]. However, no direct comparison of the effects of vitamins D_2 and D_3 has been carried out [78]. On the other hand, it has been convincingly demonstrated that vitamin D_3 significantly improves the overall level of 25(OH)D with much higher efficiency than vitamin D_2 [76, 79, 80]. It should be emphasized that this applies only to the accumulation of $25(OH)D_2$ and $25(OH)D_3$. It is not the formation of $25(OH)D_2$ and $25(OH)D_3$ from vitamins D_2 and D_3 that is of interest, but rather the physiological responses caused by the action of these two forms of vitamin D. Unfortunately, there are still very little data regarding this issue [81].

Conflict of Interests

The author declares no conflict of interests.

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