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ON THE SYNTHESIS OF VITAMIN D IN THE DARKNESS

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Abstract

Published evidence reports the existence of two routes for the transformation of 7-dehydrocholecalciferol into previtamin D_3: a photochemical route with the participation of UVB photons and another route that occurs in the darkness. Several reports appears to support the presence of these two routes in some mammals, birds, nonvascular plants (e.g.: mosses), vascular plants (e.g.: angiosperms) and lichens. The reviewed evidence suggests that in the darkness, the synthesis of vitamin D_3 follows the same scheme of the photochemical pathway, but at a reduced rate respect to the synthesis under UVB radiation. The process of vitamin D synthesis in the dark, then, may be taken as an insurance for survival, at least for mammals and birds.

The low rate of the synthesis of vitamin D_3 in the absence of light produce low concentrations of vitamin D_3 metabolites in plasma. Long term survival under these circumstances might be possible through upregulation of vitamin D receptors (VDRs). In mole rats (South African rodents that live in the dark in underground tunnels), the reduced rate of vitamin D_3 synthesis produce low levels of plasma vitamin D_3 and their metabolites 25(OH) D_3 and $1\alpha,25OH_2D_3$. The fact that Kd and β_{max} of the complex $1\alpha,25(OH)_2D_3$-VDR from the intestinal mucosa, kidneys and the Harderian glands of the mole rat Heterocephalus glaber are significantly different in each one of these tissues, is interpreted as an indicator that the VDRs are, in each tissue, adapted to the maintenance of normal physiological functions.

Key words: vitamin D, darkness, receptors, regulation, synthesis in plants.

Resumen

SOBRE LA SÍNTESIS DE VITAMINA D EN LA OSCURIDAD

Varios trabajos publicados han informado que existen dos mecanismos para la transformación de 7-dehidrocolecalciferol en previtamina D_3: uno iniciado con el auxilio de fotones UVB y un segundo que ocurre en la oscuridad, sin el auxilio de radiación ultravioleta. Una serie de publicaciones contienen información que apoya la presencia de estos dos mecanismos en mamíferos, pájaros, plantas no vasculares (musgos), vasculares (angiospermas) y líquenes. La evidencia revisada sugiere que, en la oscuridad, la síntesis de vitamina D_3 sigue

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el mismo esquema que la ruta fotoquímica y generalmente ocurre a una tasa reducida respecto de la síntesis bajo irradiación ultravioleta. La operación de la síntesis de vitamina D_3 en la oscuridad, por lo menos para mamíferos y pájaros, puede tomarse como un reaseguro de sobrevida.

La reducida tasa de síntesis de vitamina D en ausencia de luz produce bajas concentraciones en plasma de los metabolitos de la vitamina. La sobrevida saludable en estas condiciones sería posible mediante upregulation de los receptores. En las ratas topo Heterocephalus glaber (roedores sudafricanos que viven en permanente oscuridad, en túneles subterráneos), la reducida tasa de síntesis de vitamina D_3 es la causa de los bajos niveles plasmáticos de la vitamina y sus metabolitos: 25(OH)D_3 y 1α,25OH_2D_3. El hecho de que el Kd y β_{max} del complejo 1α,25$(OH)_2D_3$-Vitamina-D-Receptor del intestino, riñón y glándulas de Harder de Heterocephalus glaber *sean significativamente diferentes entre sí indicaría que los receptores se han modificado para mantener funciones fisiológicas normales en cada tejido.*

Palabras clave: *vitamina D, oscuridad, regulación, receptores, síntesis en vegetales*

Introduction

During the industrial revolution, started in Europe in the 18th century, an outbreak of Bone Deforming Disease (rickets) appeared in children. The possible relationship between the industrialization of Northern Europe and rickets was reported by Sniadecki in 1822.[1] He observed that children living in rural areas did not suffer from rickets while children born and raised in Warsaw were plagued with the disease, and attributed the high incidence of rickets, in Polish children to their lack of sun exposure. In 1919, Huldschinski[2] reported the observation that children with rickets had a dramatic healing of their disease after several months of exposure to radiation from a mercury arc lamp. Two years later Hess and Unger[3] reported that exposure to sunlight was an effective treatment for rickets. In 1935, 7-dehydrocholesterol was first isolated by Windaus *et al.* and vitamin D_3 was identified two years later by Windaus and Bock.[5] The fact that irradiation of 7-dehydrocholesterol produced vitamin D_3 was finally proven in 1978 by Esvelt *et al.*[6] through isolation and identification of vitamin D_3 by mass spectrometry. Details of the complete history of the cure of rickets by irradiation to the discovery of vitamin D_3 and the effects of its metabolites can be read in a review by De Luca.[7]

For many years, only animal products were considered as sources of vitamin D_3. Today we know that vitamin D_3 and their metabolites 25(OH)D_3 and 1α,25OH_2D_3 are synthetized as well by nonvascular plants e.g.: mosses, vascular ones e.g.: angiosperms and lichens (a symbiosis between a fungus and an alga or cyanobacteria).[8] Planktonic microalgae[9] are small microscopic photosynthetic aquatic plants that produce vitamin D and are the source of vitamin D for fish. Microalgae are important for life on earth. They contain the photosynthetic pigments chlorophyll a and c; they produce approximately half of the atmospheric oxygen and simultaneously use carbon dioxide to grow photo-autotrophically.

The physiological function of vitamin D_3 in plants is yet poorly known. Plants have calcium channels and pumps similar to those found in animals[10] and calcium ions are a core regulator of plant cell physiology.[11] Calcium is required for stimulation of growth, root initiation and promotion of germination in plants.[12] 1α,25$(OH)_2D_3$ has been shown to in-

fluence growth and calcium transport in roots of *Phaseolus vulgaris* (common bean)[14] by increasing synthesis of calmodulin.[13] a calcium-binding messenger protein found in all eukaryotic cells. The presence of tissue vitamin D_3 receptors (VDR) has been reported in *Solanum glaucophyllum* (waxyleaf nightshade).[14]

Synthesis of vitamin D in the skin

In the skin, the synthesis of vitamin D_3 has two steps (Figure 1): It starts with Δ7-dehydrocholesterol which is converted first into previtamin D_3, which later isomerizes to vitamin D_3. This two-reaction process was thoroughly investigated by Holick and coworkers.[15]

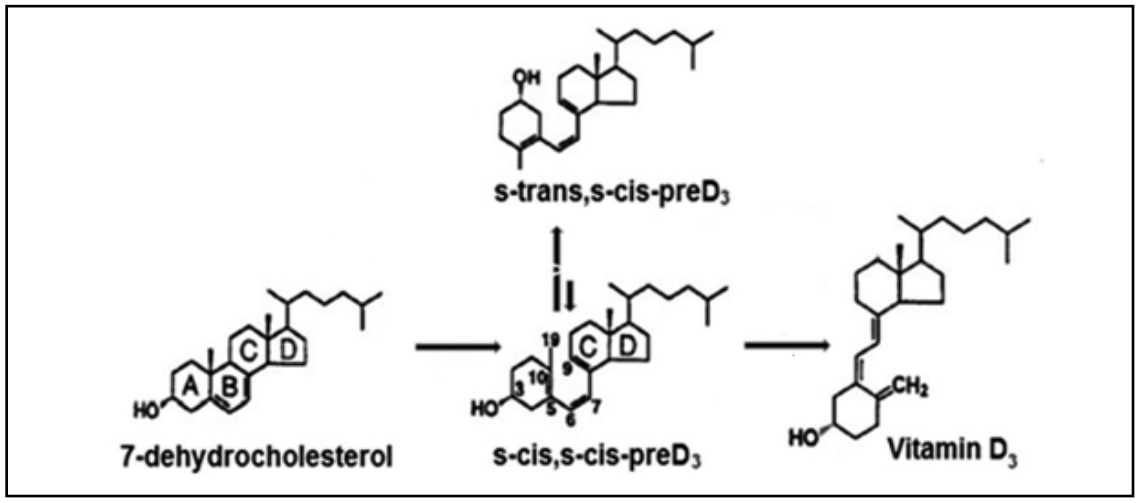

Figure 1. Photolysis of Δ7-dehydrocholesterol into s-cis,s-cis- previtamin D_3 and its thermal isomerization to vitamin D_3. Adapted from Wacker & Holick.[16]

First reaction: UVB photons excite the 7-dehydrocholesterol molecule and transforms it into a secosteroid (a steroid with a "broken" ring): the pre-vitamin D_3, a molecule that adopt two configurations: s-cis,s-cis-previtamin D_3 and s-trans,s-cis pre vitamin D_3.

Wacker and Holick[16] postulated that in an organic solvent, such as hexane, previtamin D_3 preferentially exists in the *s-trans,s-cis* conformation, which is thermodynamically more stable. The less stable conformation: *s-cis,s-cis*-previtamin D_3 in hexane at 25°C isomerizes into vitamin D_3 with a half-life time of 91 h.

Second reaction: in the skin, previtamin D_3 is maintained in the s-cis, s-cis conformation, by interaction with membrane fatty acids. The *s-cis,s-cis*-pre vitamin D_3 isomerize rapidly into vitamin D_3, with a $T_{½}$ = 8 h. Much of the information about the previtamin D_3 isomerization to vitamin D_3 was obtained from experiments using organic solvents (e.g., hexane) to assess the conversion[16,17] and assumed to reproduce the same phenomena occurring in human skin. The reaction rate of this isomerization is a direct function of temperature. Earlier studies found that this process was not affected by acids, bases, catalysts, or inhibitors of radical chain processes. Holick et al.[17] concluded that the isomerization was a rearrangement within the molecule of previtamin D_3. There is no evidence for the existence of an enzymatic process in the skin that can

convert previtamin D_3 to vitamin D_3. During the synthesis of vitamin D_3, the hydrophilic and hydrophobic interactions of the s-cis, s-cis -previtamin D_3 with the membrane fatty acids are disrupted, thereby facilitating the ejection of vitamin D_3 from the skin cell membrane into the extracellular space. It enters the circulation by binding to the serum vitamin D_3 binding protein DBP. The removal of vitamin D_3 from the skin as it is being produced changes the isomerization reaction from a reversible to an irreversible process.[15-17] This would explain the relatively rapid rise in the serum vitamin concentration after UVB exposure.

The importance of membrane microenvironments on the isomerization previtamin $D_3 \rightleftharpoons$ vitamin D_3 received further support from a kinetic study in an aqueous solution of β-cyclodextrin.[18] Cyclodextrins, a group of naturally occurring, truncated cone-shaped oligosaccharides, have an unique ability to complex a variety of foreign molecules into their hydrophobic cavities in aqueous solution including vitamin D_3. With this model, Tian and Holick[18] demonstrated that, at 5°C, the forward and reversed rate constants for the isomerization of previtamin $D_3 \rightleftharpoons$ vitamin D_3 were increased by more than 40 and 600 times, respectively, compared with those in n-hexane.

Transference of vitamin D_3 from the skin to the circulation. After previtamin D_3 is thermally isomerized to vitamin D_3, the latter is transported to the dermal capillary bed beneath the dermo-epidermal junction. The mechanism of translocation is largely unknown. In an attempt to understand this event Tian et al.[19] studied the kinetics of vitamin D_3 formation and the time course of appearance of vitamin in the circulation after exposure of chickens to UVB radiation. Their data indicate a fast rate of formation of vitamin D_3 from previtamin D_3 and a relatively fast rate of translocation from skin to circulation. By examining the time course of appearance of vitamin D_3 in circulation, they found a rapid phase of vitamin D_3 appearance from 8 to about 30 h post-irradiation and a relatively slower phase of its disappearance after the peak of vitamin D_3 in the circulation. No previtamin D_3 could be detected 1 h after UVB irradiation. Thus, only vitamin D_3 is preferentially removed from skin into circulation.

The importance of DBP in the translocation of vitamin D_3 into circulation was defined by Haddad et al.[20] They investigated the transport of skin-synthesized vitamin D_3 into circulation in seven healthy volunteers who received whole-body irradiation by assessing the time course distribution of plasma protein-bound vitamin D_3 in high (>1.3 g/ml) and low density (<1.3 g/ml) fractions after UVB irradiation. They found that plasma vitamin D_3 concentration began to increase 10 h after irradiation, peaked at 24 h, and lasted for a week in the high-density layer where all the DBP was present. These observations indicated that the endogenously photosynthesized vitamin D_3 circulates in serum bound to the DBP. The latter phenomenon differs from the orally administered vitamin D_2 [20], which is evenly distributed between the high- and low-density layers at 4, 8, and 24 h after the ingestion.

Evidence on the synthesis of vitamin D_3 in the darkness

Researchers have evaluated the synthesis of vitamin D in the darkness measuring vitamin D_3 and/or its hydroxylated metabolites as the end point of their experiments.

A. Mammals

Damara mole rats *(Fukomys damarensis)* live in underground tunnels (Pitcher et al.[21]). The plasma levels of $25(OH)D_3$ and $1\alpha,25(OH)_2D_3$ of these animals increase when exposed to sunlight or supplemented with vitamin D_3 (Table 1). These experiments demonstrated that Damara mole rats have the capacity to produce vitamin D_3 and its metabolites without the participation of sunlight.

Table 1. Plasma levels (mean ± SEM) of 25(OH)D_3 and 1α,25$(OH)_2D_3$ in controls, vitamin D_3 supplemented and exposed to sunlight Damara mole rats.

Metabolites	Controls no sunlight exposure n=10	Vit D_3 supplemented, n=5 2.5 ng/g of food eaten	Sunlight exposure n=5 15 min/day/3 weeks
25(OH)D_3, ng/ml	< 5	30±3	< 5
1α,25$(OH)_2D_3$, pg/ml	12±2	35±2	25±3

Table 2. Saturation analysis of 1α,25$(OH)_2D_3$ binding to VDRs from the intestinal mucosa, kidney, Harderian glands and skin of naked mole rats. (Sergeev et al., 1993[22])

	Tissue	β_{max} (fmoles/mg protein)	K_d (pM)
Control mole rats	Intestine mucosa	380±51.7	748±148
	Kidney	79.9±9.4[b]	183±20.2[b]
	Harderian glands	28.1±4.2[b,c]	97.0±12.2[b,c]
	Skin	<1.5	ND
Mole rats supplemented with a high dose of vitamin D_3 10 days before assay	Intestine mucosa	599±90.7[a]	813±93.9
	Kidney	158±27.6[a,b]	206±9.0[b]
	Harderian glands	32.3±5.0 [b,c]	81.7±7.6 [b,c]
	Skin	<1.5	ND

ND: not determined. Results are means ± SEM. P<0.05: [a]: from the control group, [b]: from the intestinal mucosa in the same group, [c]: from the kidney in the same group.

Table 2 has been transcribed from the paper of Sergeev et al.[22] to point out that low plasma levels of 1α,25$(OH)_2D_3$ may be compensated by upregulation of the VDRs. Table 2 displays Kd values (inversely related to of the drug affinity for the binding site) and the maximum number of binding sites per mg of receptor protein (β_{max}), of naked mole rats (*Heterocephalus glaber)*. These investigators succeeded in upregulating VDR, administering a high dose of vitamin D_3. This acute treatment increased the β_{max} from the intestinal mucosa and kidney tissue, did not affect the Kd values of VDRs of the intestinal mucosa, kidney and Harderian glands and increased the 24-hydroxylase activity in the kidneys (vitamin D_3 treated mole rats: 64.0±09.9 pmol/h/g tissue; vitamin D_3 deficient animals: 16.3±1.37 pmol/h/g tissue; P<0.05).

The fur of fur-bearing animals had been thought to be comparable to clothing in humans, which prevents vitamin D_3 synthesis in the skin during exposure to sunlight. The work of Hymøller and Jensen,[23] demonstrated otherwise. Sixteen Danish Holstein dairy cows were deprived of vitamin D_3 for 6 months by omitting the vitamin D_3 in their feed and housing them without access to sunlight. They were subjected to 4 degrees of coverage of their

bodies with a fabric that prevented vitamin D_3 synthesis in the covered skin areas. The experimental groups were: a) cows without any coverage (100% exposed body surface area), b) cows fitted with udder covers (94% exposed body surface area), c) cows fitted with horse blankets (28% exposed body surface area and d) cows fitted with both horse blankets and udder covers (24% exposed body surface area). The cows were let out to pasture daily between 10.00 and 15.00 h for 4 weeks. Blood samples were collected during the study and analyzed for their 25-hydroxyvitamin D_3 content.

The results (Figure 2) showed that uncovered cows had a higher 25(OH)D_3 concentration in plasma after 28 days of access to sunlight compared with covered cows and that the estimated peak plasma concentration of 25(OH)D_3 at the end of the experiment was inversely correlated to the body surface area covered (R^2= 0.99556, P<0.0022). It was concluded that cows, like humans, synthesize vitamin D_3 evenly over their body surface.

B. Birds

The feathers of feather-bearing birds has been thought to be comparable to clothing in humans, which prevents vitamin D_3 synthesis in the skin during exposure to sunlight. Two hypotheses predominate among those proposed for the origin of feathers: they evolved for flight or for thermal insulation (McGowan, 1989[24]).

Kale et al.[25] (Tables 3 and 4) has reported the vitamin D status of a flightless nocturnal bird, the brown kiwi (*Apteryx mantelli*). Kiwis caught in the wild had significantly low plasma levels of 25(OH)D_3 (3.0nmol/L, n=4). These birds feed mainly on earthworms (Reid et al.[26]) that contain undetectable concentrations of Vitamin D_3 (Finke[27]), with small amounts of plant matter (Reid et al.[26]). No significant differences were observed in plasma 25(OH)D_3 concentrations between those kept captive in outdoor enclosures receiving natural sunlight (19.8±10.9, n=6) and those kept in nocturnal houses receiving no supplemental UV-B light (19.7±9.5, n=6). Captive birds are fed on a mixture of in-

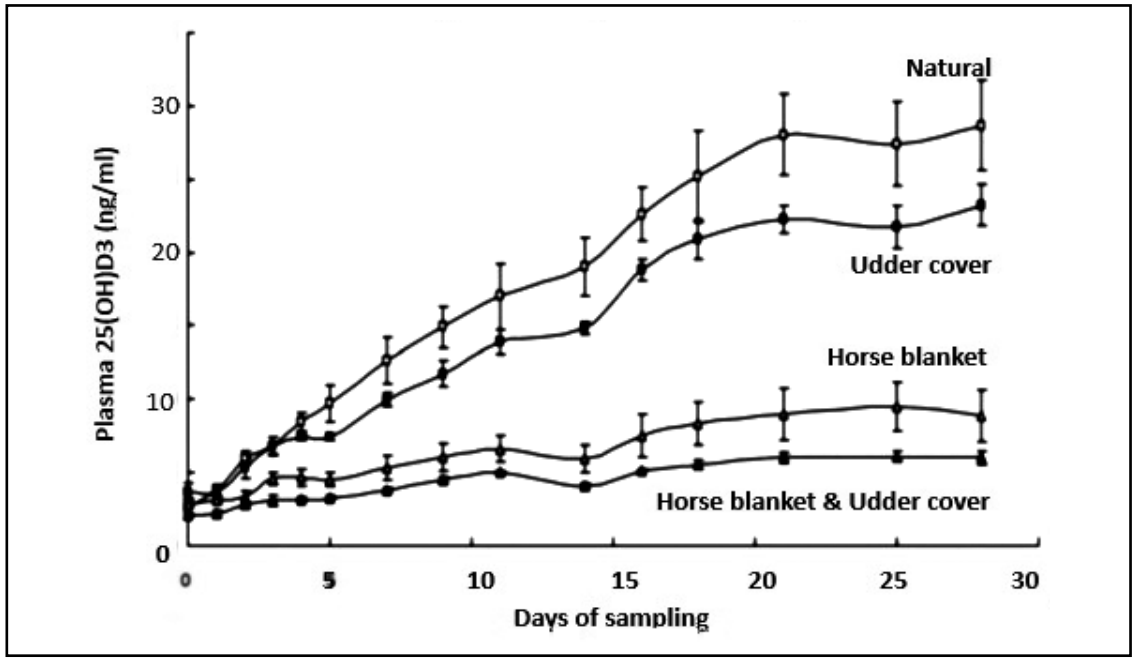

Figure 2. Plasma concentration of 25(OH) vitamin D_3 of dairy cows, after 6 months of vitamin D_3 deprivation and exposed to sunlight without (natural) and three degrees of body surface covering (see text for further details). Redrawn from Hymøller and Jensen.[23]

Table 3. Plasma 25(OH)D_3 , ng/ml (mean ± SEM) of kiwi, tuatara and New Zealand sea lions (Kale et al. 2018).[25]

Species	Plasma 25(OH)D_3
Kiwi, wild *(Apteryx mantelli)*	3.0
Tuatara, captive *(Sphenodon punctatus)*	104.2±12.8
New Zealand sea lions *(Phocarctos hookeri)*	104.4±10.0

Table 4. Concentration of vitamin D_3 (µg of vit D_3/g of fresh skin tissue) after excision of tissue samples from individuals of three species. Kale et al. 2018.[25]

Species	n	not irradiated	after 8 hours UVB*
Kiwi	4	<0.03	0.038
Tuatara	4	<0.03	0.084
Sea Lion	2	0.50	1.60

*5 J/cm^2

sects, vegetables, fruit, minced ox heart, and nutritional supplements, including a premix that contains vitamin D (Minson[28]), which explains their plasma levels of 25(OH)D_3.

Kale et al.[25] also performed *in vitro* experiments with the skins of kiwis to gain information on this site of synthesis of vitamin D_3. Skin samples were prepared in a dark room. Any fat or feathers present were removed. For each bird, two pieces of skin, two 10 cm2 pieces of skin were dissected. One piece was exposed to UVB radiation for 8 h, equivalent to 5 J/cm^2 of accumulated UV light, in a reflector box at 37 °C. The skin was moistened with phosphate-buffered saline at 1 h intervals. The control skin sample was kept wrapped in aluminum foil at 4 °C. Each sample was then cut into 5 × 5 mm pieces in a dark room, placed in a light proof bag, weighed and freeze-dried. Freeze-dried samples was extracted and analyzed for Vitamin D_3 contents. Analysis of extracts were done by reverse phase high performance liquid chromatography. Non-irradiated kiwi skins had Vitamin D_3 levels below the detection limit of the assay: <0.03 µg of vit D_3/g of fresh skin tissue. There was, however, a measurable increase in response to UV exposure, indicating the existence of the dermal synthesis pathway: 0.038 µg of vit D_3/g of fresh skin tissue.

Kiwi skins produced small but measurable amounts of Vitamin D_3 after UV irradiation. Analysis of the characteristics of their VDRs might confirm an adaptation to physiological low levels of vitamin D_3 similar to that of mole rats.

C. Plants

S. glaucophyllum is a plant native of South America. The ingestion of its leaves causes calcinosis in cattle (Worker and Carrillo 29). Early studies correlated this property to the existence in the plant of vitamin D_3, 15(OH)$_2D_3$ and 1α,25(OH)$_2D_3$ (Wasserman et al.,[30] Espareza et al.[31]). Curino et al.[32] carried out experiments with *S. glaucophyllum* tissue (callus). The term “callus” refers to an undifferentiated

plant cell mass grown on a culture medium, to produce genetically identical cells. For calli cultures, leaf explants from plants collected in the field, thoroughly disinfected, were inoculated in agar-solidified Murashige-Skoog medium supplemented with 2,4-dichlorophenoxyacetic acid and kinetin and grown at 25°C under complete darkness. Calli began to develop after 15 days of culture. After two months of subculture all the surface of culture flasks was covered with tissue. Calli grown for at least 4 months in the dark were placed in liquid nitrogen and submitted to lipids extraction. Figure 3 displays the results of Curino et al.[32], the $1\alpha,25(HO)_2D_3$ contents of *S. glaucophyllum* calli grown in the darkness *vs.* calli grown in the darkness + timed UV irradiation. The most interesting result of these experiments is the significant amount of $1\alpha,25(HO)_2D_3$ in calli grown in the darkness and not the relatively small increase of the metabolite under UV irradiation.

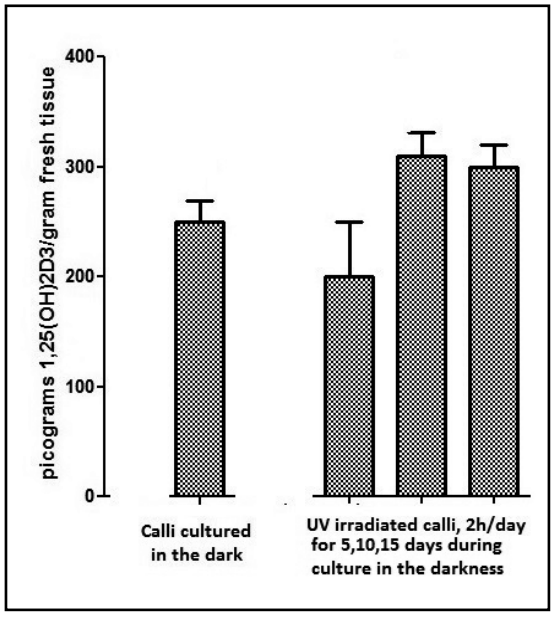

Figure 3. Tissue content of $1\alpha,25(HO)_2D_3$ of *S. glaucophyllum* cells cultured in the darkness and in the darkness+UV irradiation. Redrawn from Curino et al.[32]

Björn and Wang[33] confirmed a UVB dependent synthesis of vitamins D2 and D3 in the leaves of the tomato plant. Plants were grown in a greenhouse with or without UVB radiation 0.85 kJ plant weighted UVB radiation/m^2.per day (Table 5).

Table 5. Contents of provitamins and vitamins D_2 and D_3 µg/g of dry matter in tomato plants *Solanum lycopersicum* (Björn and Wang, 2001).[33]

	Not irradiated	Irradiated
Provitamin D_2	1.83	2.23
Vitamin D_2	0	0.087
Provitamin D_3	0.61	0.76
Vitamina D_3	0	0.28

An interesting observation is that the provitamin D_3 content of tomato leaves is not reduced by growing plants under UV-B radiation, although a substantial amount of vitamin D_3 is formed. Björn and Wang[33] assume the existence of a feedback mechanism regulating the amount of the provitamins.

The evidence reported by Jäpelt et al.[34.35] (Table 6) agrees with the above mentioned results. Note that the absence of irradiation does not cancel the synthesis of vitamin D_3 and its metabolites and that UVB irradiation increases the content of vitamin D_3 and its metabolites.

As shown by Wang et al.[36] provitamin D_2, vitamin D_2 and vitamin D_3 have been identified in the thallus of the lichen species *Cladina arbuscula.*

Inspection of Figure 4 and Table 7, indicate that the contents of vitamins D_2 and D_3 in *Cladina arbuscula* decrease as a function of the latitude. There is a significant positive correlation between the vitamins D contents

Table 6. Contents of 7-dehydrocholesterol, vitamin D_3, 25(OH)D_3 and 1,25$(OH)_2D_3$ (ng/g of dry matter) of plants cultured with a 16/8 light/dark cycle, with or without additional irradiation with an UVB source (30 min per day for 7 days). Data from Jäpelt et al.[34, 35]

Species	7-Dehydrocholesterol	Vitamin D_3	25(OH)D_3	1,25$(OH)_2D_3$
		Controls		
Solanum lycopersicum	470	1.7	<0.02	<0.1
Solanum glaucophyllum	670	3.2	0.8	<0.1
Capsicum annuum	30	<0.02	<0.02	<0.1
		UVB-irradiated		
Solanum lycopersicum	230	100	4.3	<0.1
Solanum glaucophyllum	1260	200	31	32
Capsicum annuum	30	2.9	0.5	<0.1

Table 7. Ranges of provitamin D_2, vitamin D_2 and vitamin D_3 contents (µg/g of dry weight of *Cladina arbuscula)* measured by high performance liquid chromatography, electrospray ionization mass spectrometry and UV spectrophotometry Wang et. al. 2001.[36])

Place	Approx. latitude	provitamin D_2	Vitamin D_2	Vitamin D_3
Finland	67°N	91 - 99	0.23 - 0.49	0.67 - 2.04
Sweden	56°N	106 - 120	0.43 - 0.54	1.04 - 1.24
Greece	40°N	149	0.57	1.73 - 1.84

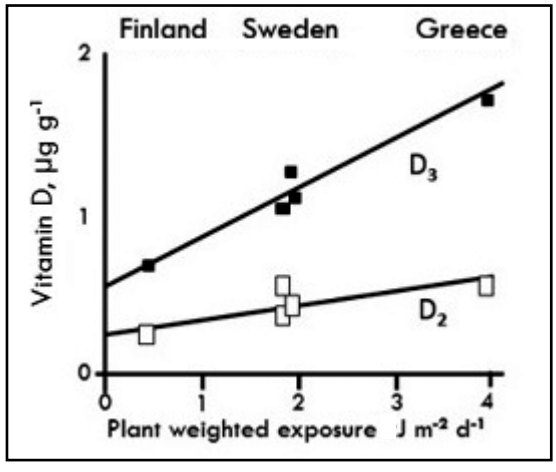

Figure 4. Left: linear relationship between the vitamins D_2 and vitamin D_3 contents of *C. arbuscula* as a function of the weighted UV exposure. Latitude of sampling places: Finland: 66-67°N, Sweden; 56°N and Greece: 40°N. Redrawn from Wang et al.[36]

and the estimated radiant energy received by lichens collected at three different latitudes. Bjõrn et al.[33] believe that the lichen might function as receptors of UV radiation. In agreement with the observations of Jäpelt et al.,[34,35] the contents of vitamins increase with the amount of radiant energy. Inspection of the Figure 4 indicate that vitamins content of Cladina are not zero at zero irradiation, which is interpreted to support the hypothesis that the synthesis of vitamin D_3 may proceed both with and without UV irradiation.

The coexistence of the two vitamins D in *Cladina* (the contents of vitamin D_3 is greater than that of D_2) may be explained by the fact that lichens are composite organisms that arise from algae or cyanobacteria living with fungi in a symbiotic relationship.

Comments on the survival of terrestrial species that live in the dark

The reviewed evidence on the synthesis of vitamin D_3 in the darkness, allows the following conclusions:

a) It uses the same components of the photochemical pathway (Figure 1).

b) It has a reduced rate respect to the synthesis under UVB irradiation.

The identification of the mechanism/s by which certain species, though lacking access to sunlight, produce enough vitamin D_3 to meet their physiological functions is an as yet unsolved matter. Assessed by actual human standards, mole rats and fruit bats[37,38] are vitamin D deficient. The natural habitats of both species exclude sunlight and their diet is herbivorous with no obvious source of vitamin D_3.

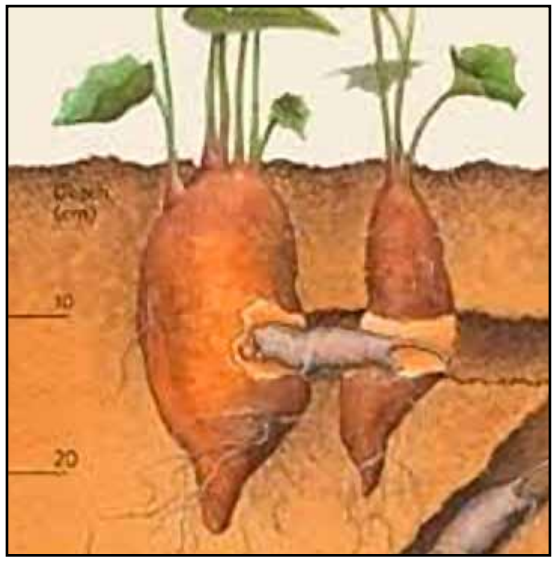

Figure 5. Scheme of the hábitat of mole rats (upper-left corner of a figure in the paper by Park and Buffenstein[39]).

Figure 5 suggests that mole rats feed on the roots of plants. This reviewer could not find a survey of the plants with subterranean enlarged organs (enlarged to store energy in the form of carbohydrates) in the areas of mole rats colonies. The amount of $1\alpha,25(OH)_2D_3$ that can be obtained from this source is probably negligible but it is as yet unknown.

With the commercial availability of $[^3H]1\alpha,25(OH)_2D_3$ as a radioligand of high specific activity, the presence of a specific binding site for $1\alpha,25(OH)_2D_3$ in low numbers (~ 100 fmol/mg protein) have been reported in *Solanum glaucophyllum* leaves, berries, stems and roots (Weissenberg et al).[40] The protein binding of $[^3H]$-vitamin D_3 has been demonstrated in *Phaseolus vulgaris* (French beans) roots.[41]

The low rate of the synthesis of vitamin D_3 in the absence of light produce low concentrations of vitamin D_3 metabolites in plasma. Long term survival under these circumstances might be possible through upregulation of VDRs. The fact that the Kd of the complex $1\alpha,25(OH)_2D_3$-VDR from the intestinal mucosa, kidneys and the Harderian glands of *Heterocephalus glaber* (south African mole rats that live in the dark in networks of underground tunnels) are significantly different for each one these tissues (Table 2),[22] is interpreted to indicate that the VDRs are, in each tissue, adapted to the maintenance of normal physiological functions.

Described in organic chemistry terms, the photochemical production of vitamin D_3 (Figure 1) is a conrotatory[A] electrocyclic[B] opening of the B-ring of 7-dehydrocholesterol to provide the triene[C] previtamin D_3, which then undergoes a thermally-induced sigmatropic[D] rearrangement to yield vitamin D_3.[42] It is of interest that *sigmatropic reactions usually do not require a catalyst*. A steric interaction between the C ring and the carbon 19 methyl group of s-cis, s-cis-previtamin D_3, could produce a conformer of previtamin D_3 energetically unfavorable and therefore less stable. As a result, a rotation around the carbon 5 and carbon 6 single bond occurs and an energetically more stable s-trans, s-cis-previtamin D_3 is formed (Figure 1). The unstable s-cis, s-cis-previtamin D_3 configuration can undergo an intramolecular hydrogen rearrangement to form vitamin D_3.

Which is/are the element/s that, in the darkness, would replace the energy of UVB photons for the opening of ring B? Hypothetically, they might be replaced by a) some as yet undetermined fraction of the electromagnetic component of cosmic rays or b) by the existence of a specific binding protein for 7-dehydrocholesterol. Experimental proof is needed to support these hypotheses.

Cosmic rays have three kinds of components: muons and hadrons (particles that have rest masses) and photons of the electromagnetic spectrum: radio waves, microwaves, infrared, visible light, ultraviolet, X-rays, and gamma rays. The ultraviolet radiation has a wavelength range 10 to 400 nm.[43,44] It appears possible that penetrating UV radiation, attenuated by the interaction with leather or feathers reach the cells containing 7-dehydrocholesterol and may contribute to the synthesis of vitamin D_3. Inspection of the action spectrum for the conversion of 7-dehydrocholesterol to previtamin D_3 (Figure 6), reveals that the optimum wavelengths for the production of previtamin D_3 lie between 295 and 300 nm with a maximum at 297 nm. It also indicate that wavelengths below 295 nm (though with lower efficiency) can break the C9-C10 bond of 7-dehydrocholesterol and produce previtamin D_3 (Figure 6).[45]

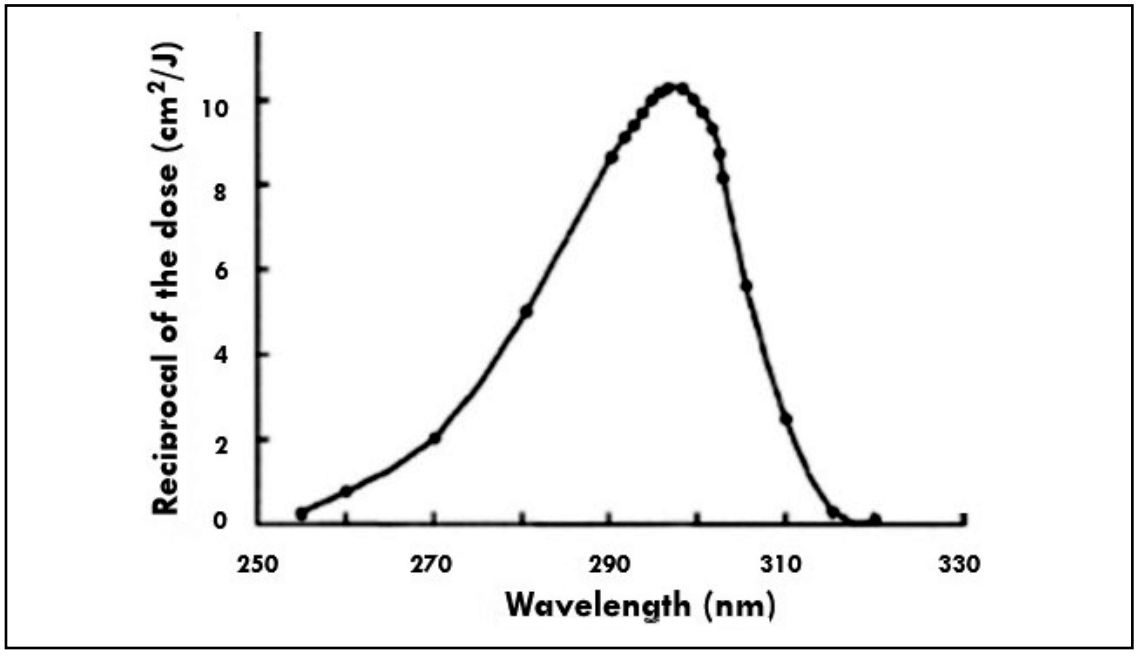

Figure 6. Action spectrum for the conversion of 7-dehydrocholesterol to vitamin D_3 in the human skin. Redrawn from MacLaughlin et al.[45]

Other possible but as yet not experimentally demonstrated mechanisms for the synthesis of vitamin D_3 in the darkness

Norman and Norman,[46] centering their attention on the opening of the B-ring of 7-dehyrocholesterol have stated: "A major unresolved problem concerns identification of the biological mechanisms by which certain animals possessing an endoskeleton but lacking access to sunlight, produce sufficient amounts of vitamin D_3 to meet their physiological needs. This includes many

sea fish, which live in the ocean below the level of sunlight penetration (10-20 m) and ...have massive quantities... of vitamin D_3 in their livers". They report that the chorismate mutase (an enzyme present in microorganisms) acting on chrorismic acid produced a retro-ene-rearrangement similar to that transforming 7-dehydrocholesterol into provitamin D_3. In simple words, an enzyme may replace the lack of light.

They also stated: "Steroid biosynthesis have been shown to proceed from squalene via a selective epoxidation and acid catalyzed olefinic cyclization. It therefore seem reasonable to suggests that such pathways have also evolved in animals lacking access to light, to catalyze the conversion of 7-dehydrocholesterol to vitamin D_3". They describe three mechanisms based on the existence enzymes demonstrated in the synthesis of steroids in vertebrates. They conclude that "It should be appreciated that the four mechanisms offered here are suggestions based upon an understanding of organic chemistry; there is no evidence to support their existence".[46]

Footnotes

A Conrotatory: An electrocyclic reaction can either be classified as conrotatory or disrotatory based on the rotation at each end of the molecule. In conrotatory mode, both atomic orbitals of the end groups turn in the same direction such as both atomic orbitals rotating clockwise or counter-clockwise.

B Pericyclic: a reaction in which bonds are made or broken in a concerted cyclic transition state. A concerted reaction is one which involves no intermediates during the course of the reaction

C triene: an unsaturated hydrocarbon containing three double bonds between carbon atoms

D sigmatropic: the name *sigmatropic* is the result of a compounding of the sigma designation from single carbon–carbon bonds and the Greek word *tropos*, meaning turn. A sigmatropic reaction in organic chemistry is a pericyclic reaction wherein the net result is one σ-bond is changed to another σ-bond in an uncatalyzed the reaction intra-molecular process. In this type of rearrangement reaction, a substituent moves from one part of a π-bonded system [alternating single and double bonds] to another part in an intra-molecular reaction with simultaneous rearrangement of the π system.

Conflicto de intereses: el autor declara no tener conflicto de intereses.

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