# MISMATCH BETWEEN THE IN VIVO AND IN VITRO VITAMIN D SYNTHESIS ACTION SPECTRA: CAUSE-AND-EFFECT RELATIONSHIP

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## MISMATCH BETWEEN THE IN VIVO AND IN VITRO VITAMIN D SYNTHESIS ACTION SPECTRA: CAUSE-AND-EFFECT RELATIONSHIP

### Summary

The matter at issue of this report is analysis of significant distinctions between the CIE in vivo and in vitro action spectra of vitamin D synthesis based on the state-of-the-art of the kinetics of previtamin D photosynthesis.

## 1 Introduction

Interest in Vitamin D has increased greatly in recent years in view of new data about its important role in reducing the risk of cancer, multiple sclerosis, and type 1 diabetes mellitus [Holick (2003)]. The vitamin D active metabolite 1,25(OH)2D is recognized as a critical hormone regulating cell growth and modulating the immune system [Mayar (1991), Holick (2002)].

Vitamin $D_3$ is produced in skin from its precursor 7-dehydrocholesterol (7-DHC) upon ultraviolet (UV) irradiation by sunlight or an artificial UV source. Vitamin D itself is biologically inactive, it is metabolized in the liver to 25-hydroxyvitamin D (25-(OH)D), and further hydroxylation occurs in the kidney and forms the very biologically active 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D) [Norman (2010)].

Assessment of the vitamin D synthetic capacity of sunlight and artificial UV sources requires an adequate action spectrum of vitamin D synthesis. That is why in 2006 CIE formed a committee (TC 6-54) to produce a standardized action spectrum (AS) for the production of previtamin D in human skin [Bouillon (2006)]. The Figure of the action spectrum published by MacLaughlin et al. [McLaughlin (1982)] was taken as the starting point. A computer algorithm was used to extract the data points from the Figure. Final action spectrum normalized to unity at 298 nm was received using further processing (interpolation and extrapolation) of these data.

As a vitamin D effective exposure is calculated by weighting a UV source spectral irradiance by the action spectrum and integrating over the wavelength interval for which the action spectrum is non-zero, obviously, a small change in the wavelength or energy of the Vitamin D action spectrum could significantly alter the interpretation of the vitamin D synthetic capacity of sunlight and/or artificial UV source.

The matter at issue of this Report is analysis of significant distinctions between the CIE in vivo and in vitro action spectra of vitamin D synthesis based on the state-of-the-art of the kinetics of previtamin D photosynthesis.

## 2 Action spectrum of vitamin D synthesis: definition and historical data

By definition, an action spectrum (AS) represents spectral dependence of the value of the biological effect initiated by monochromatic radiation of different wavelength with the same dose.

Biological effect, i.e. the production of vitamin D[1] is a two stage process. The first step is photoinduced formation of previtamin D (**Pre**) from provitamin D (**Pro**) as a result of UV photons absorption. The second step is thermo-induced conversion of previtamin D into vitamin D (**D**). Apparently, the value of previtamin D accumulated during UV exposure can serve as a measure of biologically effective (‘anti-rachitic’) UV dose.

As the rate of previtamin D accumulation at the initial stage of UV irradiation is primarily dependent on the efficiency of photon absorption by provitamin D molecule, one would expect correlation of the action spectrum of vitamin D synthesis with the absorption spectrum of provitamin D.

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[1] The terminology vitamin D is employed here in a general sense. Vitamin $D_2$, or ergocalciferol ($C_{28}H_{44}O$), is synthesized upon UV irradiation mainly in plants from ergosterol (provitamin $D_2$), similar to vitamin $D_3$, or cholecalciferol ($C_{27}H_{44}O$) which is photochemically produced in animal and human skin from 7-dehydrocholesterol (7-DHC, provitamin $D_3$). It is significant that basic monomolecular isomerizations of the two steroid species occur in perfect analogy.

Earlier studies on the quantitative efficiency of different parts of the ultraviolet spectrum for the albino rats [Knudsen (1938)] showed that the anti-rachitic curve follows somewhat the absorption curve of provitamin D. However, the action spectrum of previtamin $D_3$ photosynthesis measured with UV irradiation of human skin [MacLaughlin (1982)] did not show any correlation with the absorption spectrum of provitamin D (Figure 1).

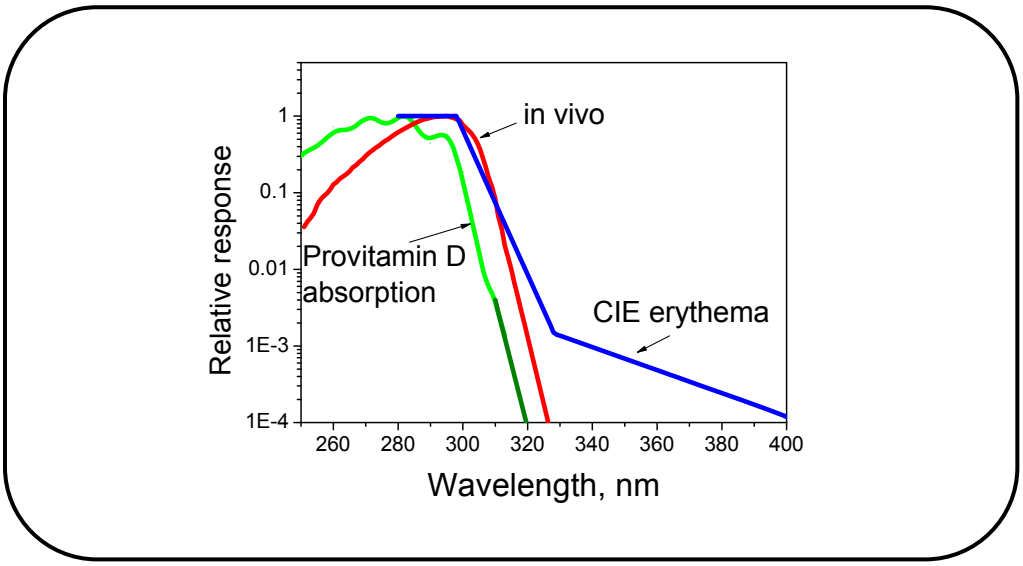


**Figure 1. The absorption spectrum of 7-DHC (extrapolated from 310nm) and the CIE Vitamin D action spectrum in human skin in comparison with the CIE erythema action spectrum [McKinlay (1987)]**

Several additional features in the graphs presented in Figure 1 are worthy of notice:

1. Significant distinction between CIE erythema AS and both 'Vitamin D' curves in the UVA spectral range,
2. Opposite relation of the two 'Vitamin D' spectra to the CIE erythema action spectrum around 310nm,
3. Difference in the wavelengths of maximum in the Vitamin D AS (295nm) and in the provitamin D absorption spectrum (282 nm),
4. Starting from 305nm to the longer wavelengths the difference between the Vitamin D *in vivo* and vitamin D *in vitro* action spectra exceeds more than one order of magnitude!

As a result, the calculated vitamin D effective ('antirachitic') irradiance of sunlight at low elevation angle is almost 50 times higher for the *in vivo* AS than for the *in vitro* one. Moreover, within 300-320 nm. the calculated 'antirachitic' irradiances demonstrate a great difference relative to erythemal irradiance: the 'antirachitic' irradiance *in vivo* ranks over, whereas the *in vitro* one represents only a small part of erythemally effective irradiance. (Figure 2)

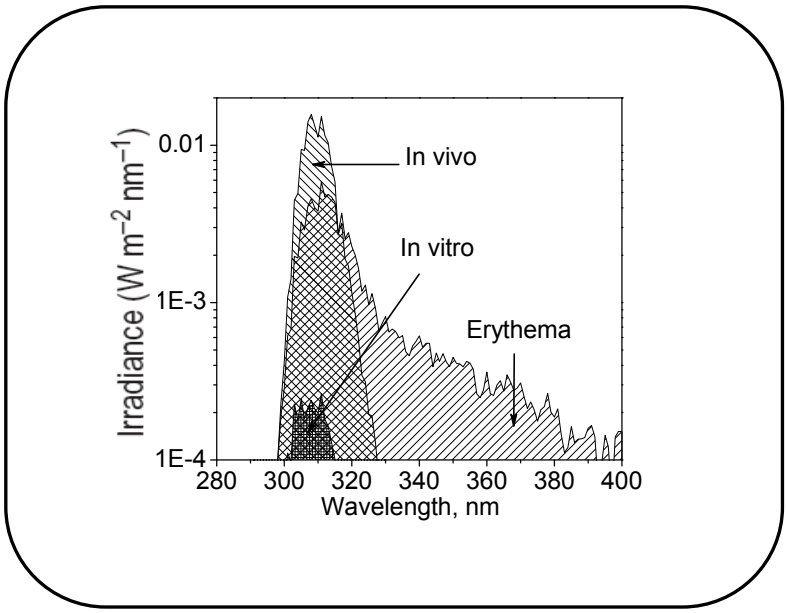


**Figure 2. Calculated vitamin D effective irradiance (*in vivo* and *in vitro*) in relation to erythemally effective irradiance for sunlight in Kiev (50,23N, 30,32E) on October 22 (SZA = 61,04);**

Taking into account that for albino rats AS bears similarities to the absorption spectrum of provitamin D molecule with maximum at 282nm, but the AS for human skin with its maximum at 295nm is in no way related to the absorption spectrum of provitamin D, it may be suggested that human skin transmission is responsible for the remarkable difference between the *in vivo* and *in vitro* action spectra of vitamin D synthesis. Hence, properly accounted for, skin transmission might be able to eliminate the difference between the *in vivo* and *in vitro* action spectra (if no photocatalysts in skin provide the antenna effect described in [Nowakowska (1997)] and summarized in the conclusion of this report). To check this assumption the absorption spectrum of 7-DHC was multiplied with the transmission spectrum of stratum cornea measured by Weil et al. (1984) (Fig.3).

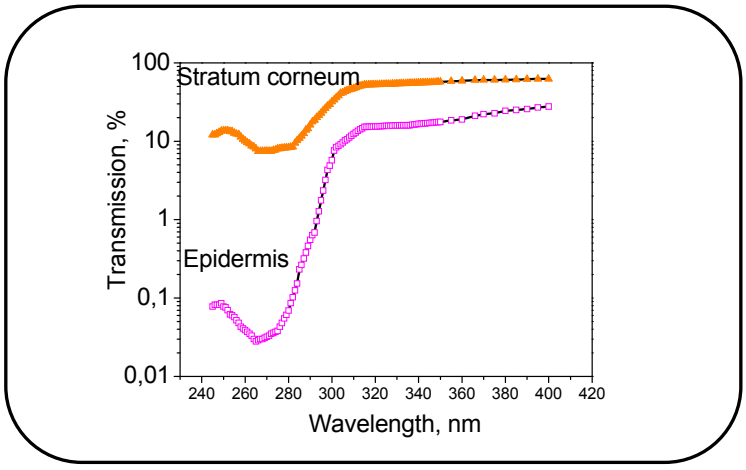


**Figure 3. Transmission spectra of stratum cornea and epidermis**

The result shown in Figure 4a) demonstrates that this procedure just increased the difference between the *in vitro* and *in vivo* action spectra. But this difference remarkably tends to diminish due to the spectra normalization at maximum 295nm which artificially brought together the long-wave wings of the action spectra (Fig.4b).

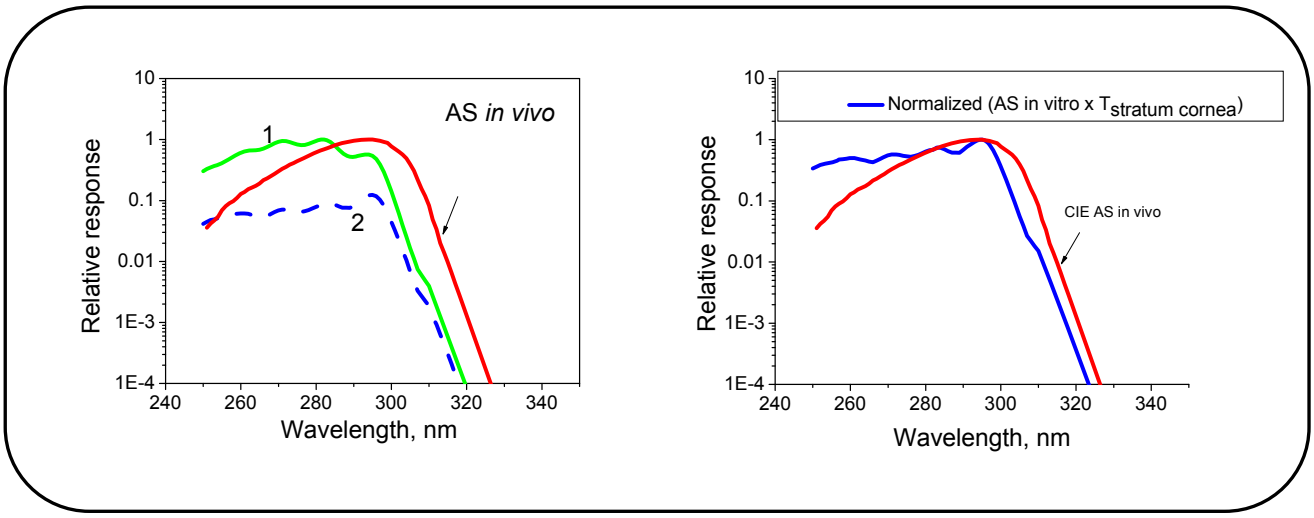


**Figure 4. (a) The transparency effect of stratum cornea (2) on the action spectrum of vitamin D synthesis *in vitro* (1); (b) Effect of the normalization procedure at 295 nm.**

To appreciate the significance of this discrepancy we calculated biologic (antirachitic) irradiance of sunlight using the CIE *in vivo* Vitamin D action spectrum and the skin-corrected AS *in vitro* for summer sunlight (Fig.5). As one can see, even at high elevation angle the difference is still profound.

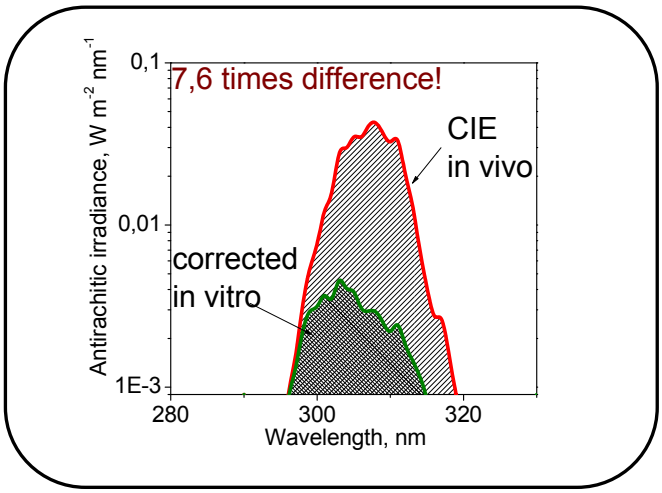


**Figure 5. Antirachitic irradiance of sunlight (SZA = 27$^0$, clear sky) calculated with the CIE Vitamin D AS *in vivo* and with the skin-corrected AS *in vitro* .**

From the above it follows that correction for human skin transmission can not bridge the gap between the *in vivo* and *in vitro* action spectra of vitamin D synthesis. We shall deal with this subject in the next chapter in which photochemistry of provitamin D is described on the basis of present knowledge [Terenetskaya (2008, 2011)].

## 3 Main features of provitamin D photochemistry: the well-known and little-known wavelength effects

The complex network of vitamin D synthesis *in vitro* has been intensively studied by E. Havinga and co-authors [Havinga(1973), Abillon (1973), Jacobs (1979)]. UV irradiation of provitamin D within its absorption band (240-315nm) yields previtamin D by hexadiene ring

opening (Fig.6). Once formed, previtamin D converts into vitamin D by the thermoinduced intramolecular hydrogen shift[2].

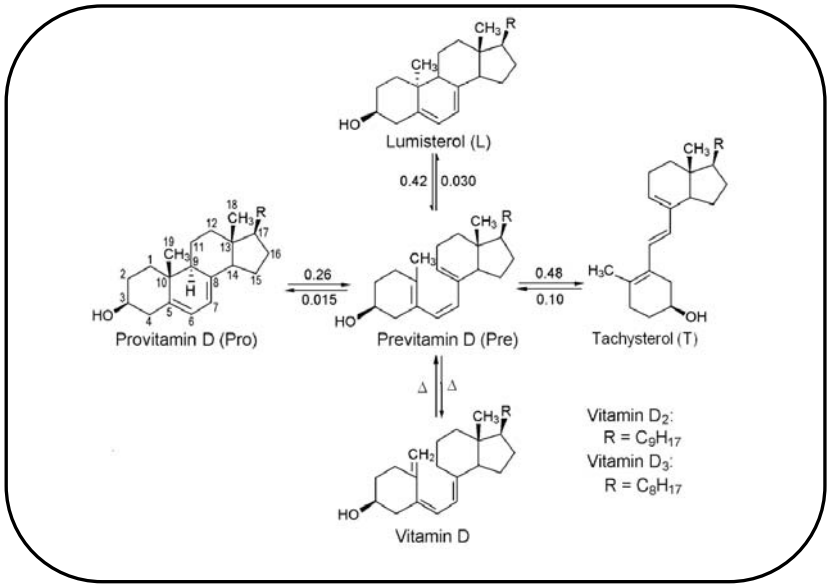


**Figure 6. Commonly used scheme of vitamin D synthesis**

The photoreaction is considerably complicated by the side photoconversions of previtamin D which has an absorption band lying in the same spectral range as is provitamin D (Fig.7). As a result, upon UV irradiation previtamin D undergoes a series of the photoconversions: reversible *cis-trans* isomerization into tachysterol (**T**) exhibits the highest quantum yield, and reversible ring-closures into initial provitamin D or its diastereomer lumisterol (**L**) are less probable. Hereupon UV irradiation of provitamin D gives rise to the mixture of four photoisomers (**Pro**, **Pre**, **T** and **L**) at a temperature preventing formation of vitamin D.

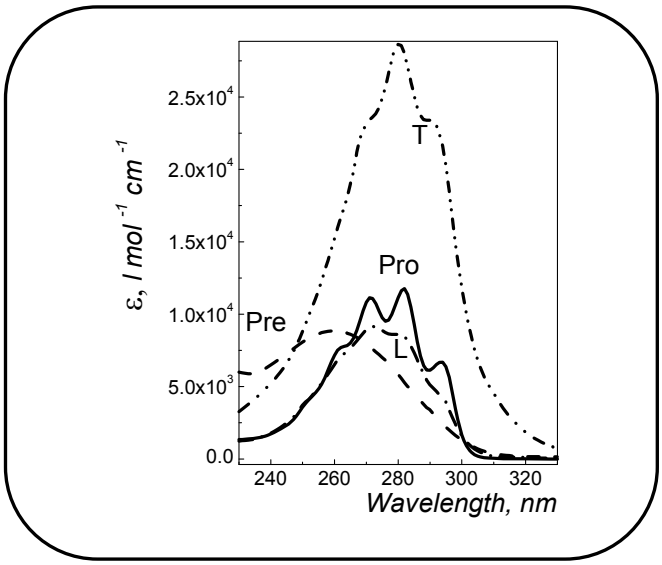


**Figure 7. UV absorption spectra of provitamin D and its main photoisomers.**

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2 At 20C, *in vitro*, it takes 20 hours to form 10% of vitamin D from previtamin D, and Pre<=>D equilibrium is established in 30 days. At 40C these times reduce to 2,3 hours and 3,5 days correspondingly [Hanewald (1961)].

In due course, a dynamic equilibrium between the photoisomers, so-called **photostationary state** (PS), is established. Previtamin D and tachysterol usually dominate in the photostationary state, and the **Pre**/**T** ratio is strongly dependent on the irradiation wavelength [Havinga (1973)]. It has been found that irradiation at 254 nm ensures high yield of tachysterol, and its amount decreases as the initiation radiation wavelength becomes longer. **This wavelength effect known long ago is caused mainly by the different absorbencies of the photoisomers involved in the reaction network**.

In spite of the fact that irreversible photoconversions into so-called “over-irradiation products” toxisterols have been known for a long time [Abillon (1973), Boomsa (1977)], it is usually assumed that due to the low quantum yield toxisterols accumulate on prolonged exposure after complete conversion of the initial provitamin D. Therefore, the irreversible channel does not as a rule appear on the reaction scheme, and the presence of toxisterols is disregarded when analyzing the photoisomer mixture, i.e. the total concentration of the four main photoisomers is taken as 100%.

Inadequacy of the photostationary state approximation has been revealed when we used laser initiation of provitamin D photoisomerization and spectral monitoring of the photoreaction kinetics [Terenetskaya (1988), Bogoslovsky (1989), Terenetskaya (1991)]. Transformation of the spectrum of 7-DHC with exposure showed unexpected behavior (Fig.8). In conflict with the generally accepted idea on the establishment of a photostationary state the absorbance near to maximum at 282nm dropped exponentially from the very beginning of laser irradiation indicating irreversible photodegradation. In addition, comparison of the final spectrum in Fig.8 with the spectra of the main photoisomers showed that the resultant photoproduct was none of those given in Fig.7.

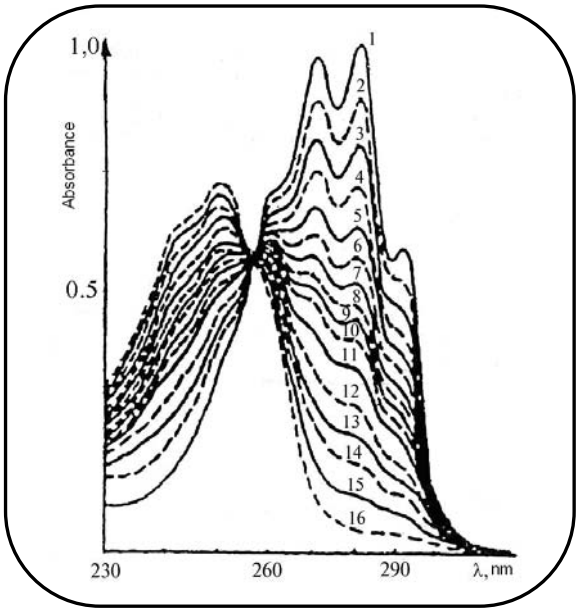


**Figure 8. Transformation of initial spectrum of 7-DHC in ethanol (1) as a result of pulsed XeCl laser irradiation at 308 nm (P = 300 mW, f = 20 Hz) during 0,5 min (2), 1 min (3), 1,5 min (4), 2 min (5), 2,5 min (6), 3 min (7), 3,5 min (8), 4 min (9), 4,5 min (10), 5 min (11), 6 min (12), 8 min (13), 10 min (14), 12 min (15) and 16 min (16).**

Hence our experiments have demonstrated that the usual range of reversible photoreactions, shown in Fig.6, was blocked by the irreversible photoconversions with low quantum yield, and the concept of their secondary role is no longer correct when the photoreaction is initiated by laser radiation at 308 nm. We were able to detect this feature due to use of UV absorption spectroscopy in contrast to the widely used chromatographic analysis. Additional experiments

showed that high intensity of laser initiation is not responsible for such unconventional course of the photoreaction,

The reasons for changes in the photoreaction pattern as a result of long-wavelength irradiation were identified by numerical calculations of the reaction kinetics in accordance with two schemes: that including only reversible photoconversions (Fig.7) and that supplemented by the Pre→ Tox irreversible photoreaction channel (Fig.9).

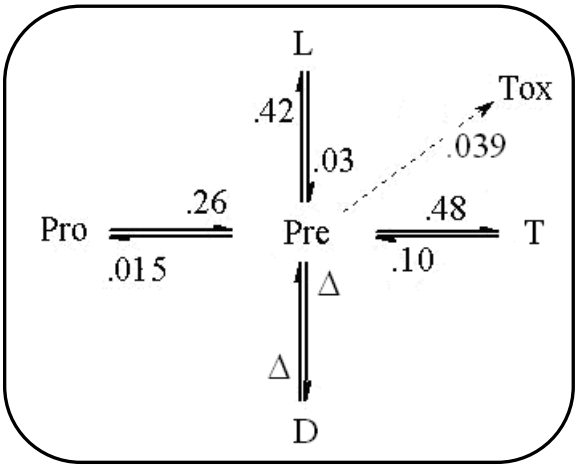


**Figure 9. Schematic representation of provitamin D photoisomerization with regard to the irreversible channel.**

The scheme with the reversible photoreactions only is described by the following familiar system of differential equations:

$$\begin{cases} \dfrac{dc_1}{dt} = \dfrac{I}{\sum_{i=1}^{4}\varepsilon_i c_i}(\varphi_{21}\varepsilon_2 c_2 - \varphi_{12}\varepsilon_1 c_1); & (3.1) \\ \dfrac{dc_2}{dt} = \dfrac{I}{\sum_{i=1}^{4}\varepsilon_i c_i}[(\varphi_{12}\varepsilon_1 c_1 + \varphi_{32}\varepsilon_3 c_3 + \varphi_{42}\varepsilon_4 c_4) - (\varphi_{21} + \varphi_{23} + \varphi_{24})\varepsilon_2 c_2]; & (3.2) \\ \dfrac{dc_3}{dt} = \dfrac{I}{\sum_{i=1}^{4}\varepsilon_i c_i}(\varphi_{23}\varepsilon_2 c_2 - \varphi_{32}\varepsilon_3 c_3); & (3.3) \\ \dfrac{dc_4}{dt} = \dfrac{I}{\sum_{i=1}^{4}\varepsilon_i c_i}(\varphi_{24}\varepsilon_2 c_2 - \varphi_{42}\varepsilon_4 c_4). & (3.4) \end{cases}$$

Here, the indices 1,2,3, and 4 denote **Pro**, **Pre**, **T**, and **L**, respectively; $c_i$- are the concentrations; $\varepsilon_i$ are the molar extinction coefficients; $\varphi_{ij}$– are the quantum yields of $i \to j$ photoconversions. It is assumed that the principle of additivity of the optical densities applies, so that the intensity of the radiation absorbed by the $i$-th component of the mixture is

$$I_i = I_0 \frac{\varepsilon_i c_i}{\sum_{i=1}^{n}\varepsilon_i c_i}(1 - 10^{-\sum_{i=1}^{n}\varepsilon_i c_i d}) = I \frac{\varepsilon_i c_i}{\sum_{i=1}^{n}\varepsilon_i c_i}, \quad (3.5)$$

where $I = I_0(1-10^{\sum_{i=1}^{n}\varepsilon_i c_i d})$ is the intensity of the radiation absorbed by a system with $n$ components; $d$ is the thickness of the irradiated layer; $I_0$ is the intensity of the incident radiation.

The scheme including the **Pre**→**Tox** (2→ 5) irreversible photoconversion channel can be described if the second equation in the above system is supplemented by an appropriate term:

$$\frac{dc_2}{dt} = \frac{I}{\sum_{i=1}^{4}\varepsilon_i c_i}[(\varphi_{12}\varepsilon_1 c_1 + \varphi_{32}\varepsilon_3 c_3 + \varphi_{42}\varepsilon_4 c_4) - (\varphi_{21} + \varphi_{23} + \varphi_{24})\varepsilon_2 c_2 - \varphi_{25}\varepsilon_2 c_2]. \quad (3.6)$$

Then the rate of change of the concentration of **Tox** becomes

$$\frac{dc_5}{dt} = \frac{I}{\sum_{i=1}^{4}\varepsilon_i c_i}\varphi_{25}\varepsilon_2 c_2 \quad (3.7)$$

(The absorbance of radiation by **Tox** and, consequently, their phototransformations are ignored). In these calculations we used numerical values of $\varepsilon_i$ from Sternberg [Sternberg (1960)], $\varphi_{ij}$ from Jacobs [Jacobs (1979)] and $\varphi_{25}$ from Abillon [Abillon (1973)].

The results of a numerical analysis of the kinetics of provitamin D photoisomerization in accordance with the above two schemes at $\lambda_{irr}$=295nm and 308nm are shown in Fig.10. A comparison of Fig.10a) and 10c), corresponding to the reversible photoreaction scheme shows different compositions of the photoisomer mixture in the PS state for different irradiation wavelengths: in the case of $\lambda_{irr}$=282nm the dominant products are **Pre** and **T**, whereas at $\lambda_{irr}$=308nm the concentrations of all four photoisomers are comparable.

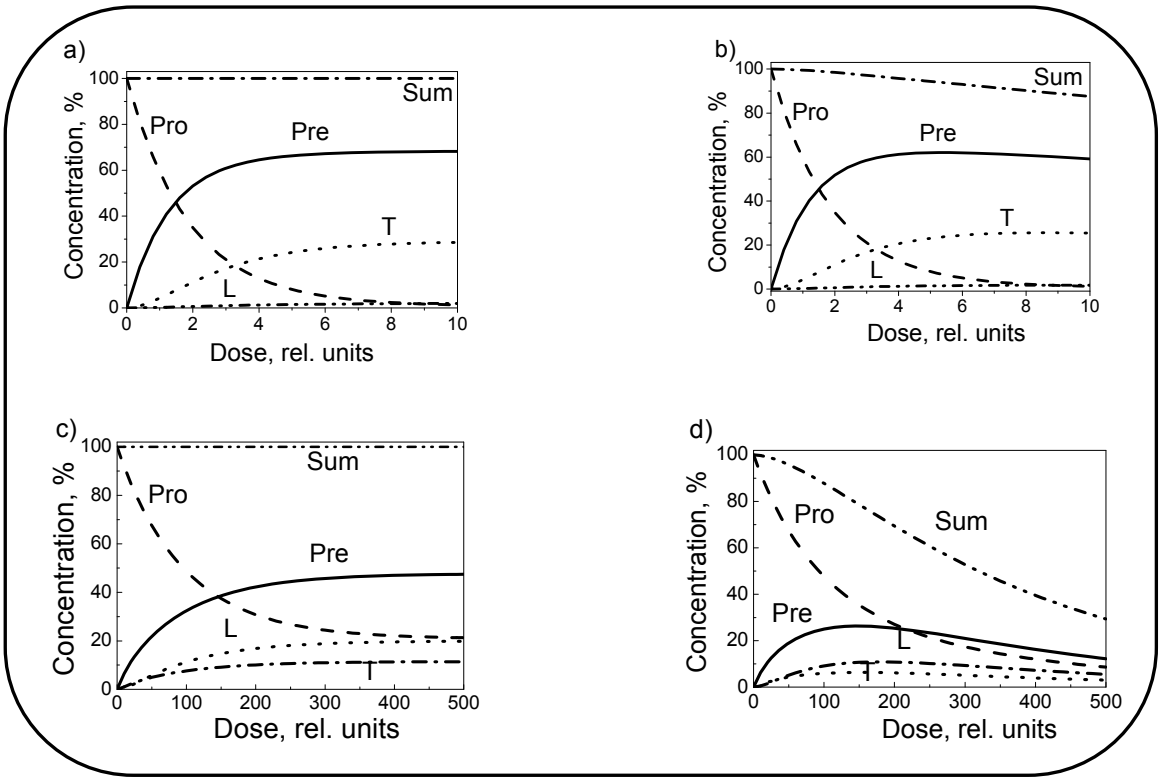


**Figure 10. Simulated kinetics of provitamin D photoisomerization upon UV irradiation at 295nm (a,b) and 308nm (c,d) without the irreversible channel (a,c) and with due regard to the irreversible photoconversions Pre → Tox (b,d). 'Sum' is summary concentration of the four main photoisomers.**

A comparison of Figs 10a) and 10c) with Figs 10b) and 10d), where the kinetics are shown for the same irradiation conditions but allowing for the **Pre → Tox** irreversible channel, shows that inclusion of the irreversible channel has little effect on the kinetics upon irradiation at 295nm: there is only a slight reduction in the concentrations of Pre and T in the PS state, and neglect of the small amount of **Tox** accumulated up to the moment of establishments of the PS state does not result in significant error in the concentration analysis.

But at $\lambda_{irr}$=308nm an allowance for the **Pre→Tox** conversions changes dramatically the reaction kinetics: **Tox** accumulate rapidly from the earliest stage of irradiation significantly reducing (by a factor of almost 2) the maximum concentrations of **Pre** and **T,** so that the content of **Tox** by that moment becomes comparable with the concentration of **Pre** and exceeds the concentrations of the remaining photoisomers.

Similar computer simulations were then made for different excitation wavelengths, and they showed that account for the channel of irreversible **Pre→Tox** photoconversions had little effect on the kinetics for $\lambda_{irr}$ in the range of 270-295nm.

Thus, the anomalous kinetics of the photoreaction during irradiation in the region of $\lambda_{ir}$ > 300 nm required explanation. It was necessary to determine why the establishment of a PS state was not observed while the initial provitamin D converted irreversibly into toxisterols.

### 3.1 Simplified model of reversible photoconversions with a weak irreversible channel

For a better understanding of the reasons for the increase effectiveness of the irreversible **Pre→Tox** channel in the region of 295-305nm we have examined the simplified model [Terenetskaya (2008)].

Let us suppose that there are two photoisomers **A** and **B** with overlapping absorption spectra (Fig.11), between which reversible photoconversions **A**↔**B** are possible with equal quantum yields $\varphi_{AB} = \varphi_{BA} = 0.5$. The initial concentrations are $C_A = 1$ and $C_B = 0$. Since the photoreaction is reversible, a photostationary state is clearly realized at some time after the beginning of irradiation in which the concentrations of **A** and **B** adopt specific values depending on the irradiation wavelength.

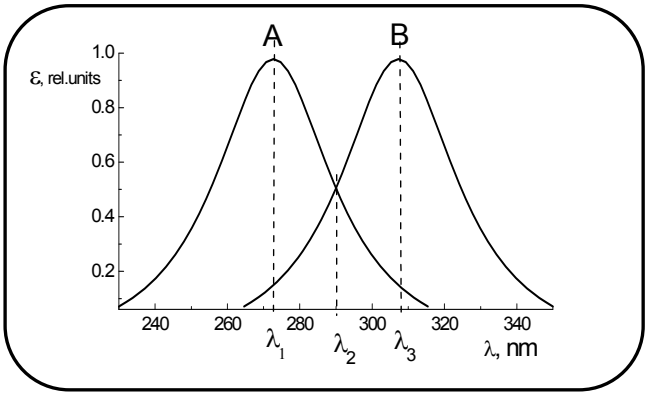


**Figure 11. Model absorption spectra of the photoisomers A and B.**

We examine three different cases of irradiation at the wavelengths with the following ratios of the absorption of **A** and **B**:

at $\lambda_1$: $\varepsilon_A/\varepsilon_B = 10$,

at $\lambda_2$: $\varepsilon_A/\varepsilon_B = 1$,

at $\lambda_3$: $\varepsilon_A/\varepsilon_B = 0.1$.

Then the concentrations of the photoisomers in the photostationary state are related as:

$$\frac{C_A}{C_B} = \frac{\varepsilon_B \varphi_{BA}}{\varepsilon_A \varphi_{AB}} \tag{3.8}$$

Hence, if $\varphi_{AB} = \varphi_{BA}$, the ratios of the absorption parameters at the three selected wavelengths predetermine the following concentrations of **A** and **B** in the photostationary state upon monochromatic irradiation:

at $\lambda_1$: $C_A/C_B = 0.1$,

at $\lambda_2$: $C_A/C_B = 1$, (3.9)

at $\lambda_3$: $C_A/C_B = 10$.

Now let us assume that an irreversible photoreaction **B** → **X** is possible from **B** with the quantum yield 10 times smaller than the quantum yields of the reversible photoconversions (i.e., $\varphi_{BX} = 0.05$),.

For the photoreaction **A**↔**B**→**X** the variation of the concentrations of **A** and **B** with the irradiation time is described by the following system of equations:

$$\frac{dC_A}{dt} = Il(\varepsilon_B \varphi_{BA} C_B - \varepsilon_A \varphi_{AB} C_A), \tag{3.10}$$

$$\frac{dC_B}{dt} = Il[\varepsilon_A \varphi_{AB} C_A - (\varepsilon_B \varphi_{BA} - \varepsilon_B \varphi_{BX})C_B] \tag{3.11}$$

where ***I*** is the irradiation intensity; *l* is the thickness of the irradiated layer.

In this case the ratio of the rates $V_{BX}$ for the decomposition of **B** (the effectiveness of the irreversible channel **B→X**) and $V_{AB}$ for the formation of B (by the phototransformation **A→B**) is determined by the equation

$$\frac{V_{BX}}{V_{AB}} = \frac{\varepsilon_B \varphi_{BX}}{\varepsilon_A \varphi_{AB}}. \tag{3.12}$$

The simplest calculation shows that the effectiveness of the irreversible channel for the three wavelengths will differ substantially:

At $\lambda_1$: $\frac{V_{BX}}{V_{AB}} = \frac{1}{10} \cdot \frac{0.05}{0.5} = 0.01$, (3.13)

at $\lambda_2$: $\frac{V_{BX}}{V_{AB}} = 0.1$, (3.14)

at $\lambda_3$: $\frac{V_{BX}}{V_{AB}} = 1$. (3.15)

As evidenced by comparison of the expressions (3.13)-(3.15), the secondary role of the irreversible channel during irradiation at $\lambda_1$ can increase substantially with initiation of the photoreaction at other wavelengths even in spite of the low value of its quantum yield. Thus, during irradiation at $\lambda_3$ the rate of the irreversible decay **B→X** becomes equal to the rate of formation of **B** from **A**. As a result, almost direct photoconversion **A→X** occurs, and the accumulation of **B** here is greatly reduced.

The situation described above (with identification of the hypothetical photoisomers **A**, **B**, and **X** with **Pro**, **Pre**, and **Tox**) is realized at the long-wave edge of the absorption spectrum of Pro from the long-wave side of the intersection of the spectra of **Pro** and **Pre**

As it is seen in Fig.12, the calculated rate of the irreversible photoreactions **Pre→Tox** in relation to the rate of formation of previtamin D increases greatly when irradiation is provided at the long-wave edge of the absorption spectrum.

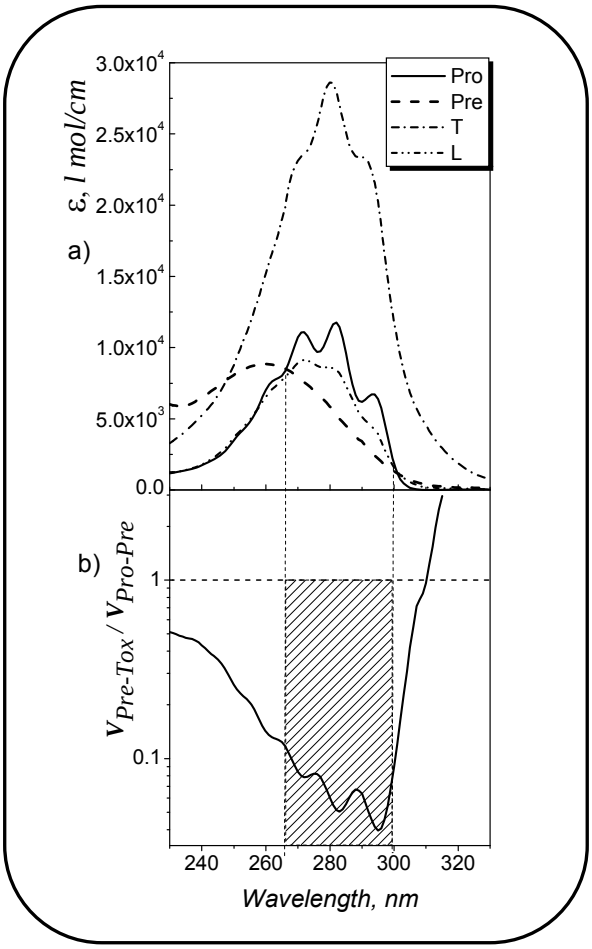


**Figure 12. The absorption spectra of the four main photoisomers of provitamin D (a) and the calculated ratio of the rate of the irreversible photoreactions Pre→Tox to the rate of formation of the previtamin D Pro→Pre (b). The shaded region shows the range of wavelengths 266-300 nm in which the PS approximation fits.**

It is seen that only in the wavelength range 266-300nm between the isosbestic points, at which the absorption coefficients of **Pro** and **Pre** are equal to each other, the rate of the irreversible phototransformations of previtamin D into the toxisterols is ten times less than the rate of its formation from provitamin D. Therefore, only at irradiation in this spectral region, in which the absorption coefficient of **Pro** is higher than for **Pre**, does the PS approximation adequately describe the kinetics of the photoreaction, and the traditional disregard of the irreversible channel is justified.

It is clear from the above that the usual concept of the unimportance of the irreversible photoreactions breaks down during long-wave irradiation. Under these conditions the toxisterols stop conforming to the name “overirradiation products” since they are accumulating at the initial stage of irradiation and their rapid accumulation restricts the growth in the concentrations of the main photoisomers. As a result, the maximum attainable concentrations of **Pre** and **T** differ radically from those calculated in terms of the PS approximation.

As evidenced by our calculations, if the total concentration of the four photoisomers **Pro, Pre**, **T**, and **L** has traditionally been taken as equal to 100% and the **Tox** content has been disregarded in concentration analysis, this introduces error which increases sharply as the irradiation wavelength is shifted toward the red region from 300 nm, and an adequate analysis should take into account the mixture photodegradation (Fig.10d). Accumulation of toxisterols that in fact represent 13 photoisomers [Boomsma (1977)], poses major problem for the commonly used method of high performance liquid chromatography (HPLC).

These conclusions are supported fully by the experimental research [Terenetskaya (2011)] carried out using specially designed spectrophotometric analysis, which does take into account the photoisomeric mixture degradation of [Terenetskaya (2000)].

## 4 Precise measurement of an in vitro action spectrum of vitamin D synthesis

In theory, to determine the biologically effective irradiance, the spectrum measured by a spectroradiometer is to be weighted (integrated over the wavelengths) according to the action spectrum of a specific biological effect [Horneck (1995)]. As noted above, the CIE erythema action spectrum is the most common choice in the so-called 'weighted spectroradiometry' [McKinlay (1987)].

$$E_{eff} = \int E_{\lambda}(\lambda) * S_{\lambda}(\lambda) d\lambda$$

Here $E_{\lambda}(\lambda)$ - solar spectral irradiance [$Wm^{-2}nm^{-1}$], $S_{\lambda}(\lambda)$ - action spectrum [relative units], λ-wavelength [nm]. Integration of the biologically effective irradiance $E_{eff}$ over the time gives the biologically effective dose $H_{eff}$ [$Jm^{-2}$]:

$$H_{eff} = \iint E_{\lambda}(\lambda, t) * S_{\lambda}(\lambda) d\lambda dt$$

Biological dosimeters use the simplest biologic systems (bacteria, spores, biomolecules) and directly integrate UV radiation during an exposure according to the action spectrum of the photobiologic effect involved [Horneck (1997)].

Contrary to the majority of biological dosimeters, for which there is an imperfect understanding of the photobiological process details (especially when such a complex system as living cells is used), in the case of the D-dosimeter the photoreaction mechanism and physical parameters are known in detail, and the adequate mathematical model sets up a correspondence between the physical ($J/m^2$) and biological (previtamin D concentration) units of the accepted UV dose.

An original method based on an *in vitro* model of vitamin D synthesis (the so-called 'D-dosimeter') had been introduced for the UV dosimetry *in situ* using original spectrophotometric concentrational analysis. [Galkin (1999)].

Determination of the vitamin D action spectrum *in vitro* was performed at the Belgian Institute for Space Aeronomy [Bolsee (2000)] using UV irradiation from a xenon arc lamp with a number of narrow-band filters (Δλ =2.5nm). A solution of 7-DHC in ethanol (C =20μg/ml, V=2ml) was irradiated in a standard rectangular cuvette (Hellma) of 0.5cm thickness. During the time when the cuvette with the provitamin D solution was exposed, the incident UV radiation was measured by the spectroradiometer Spex Model 1672M.

Absorption spectra of provitamin D before and after several exposures were recorded, and the photoisomer concentrations were derived from the spectra by computer processing with the original program taking into consideration the irreversible photoconversions **Pre→Tox**. The concentrations of accumulated previtamin D against the irradiation time were plotted, and the required energy doses for 5% of previtamin D were calculated by using linear portion of the calibration curves. The results are presented in Table 1 where the physical and erythemal doses to achieve 5% accumulation of previtamin D are shown for the six wavelengths.

**Table 1. The measured Antirachitic, Erythemal and Physicsl doses at different wavelengths**

| Wavelength (nm) | 260 | 270 | 280 | 290 | 300 | 310 |
|---|---|---|---|---|---|---|
| Antirachitic dose (%PreD) | 5 | 5 | 5 | 5 | 5 | 5 |
| Erythemal dose (MED) | 0.28 | 0.21 | 0.20 | 0.48 | 0.70 | 3.87 |
| Physical dose ($J/m^2$) | 55 | 42.7 | 40 | 95 | 205 | 10400 |

The sharp increase in the physical dose and MED required for accumulation of 5% previtamin D at 310nm is of special interest. The increase of MEDs (Fig.13) is in conflict with the relationship between the CIE erythema and Vitamin D *in vivo* action spectra (as it was mentioned above (see Figs.1,2) Besides, it is clear from Fig.13 that the relationship between MED and previtamin D (vitamin D) formation is not constant, but wavelength dependent.

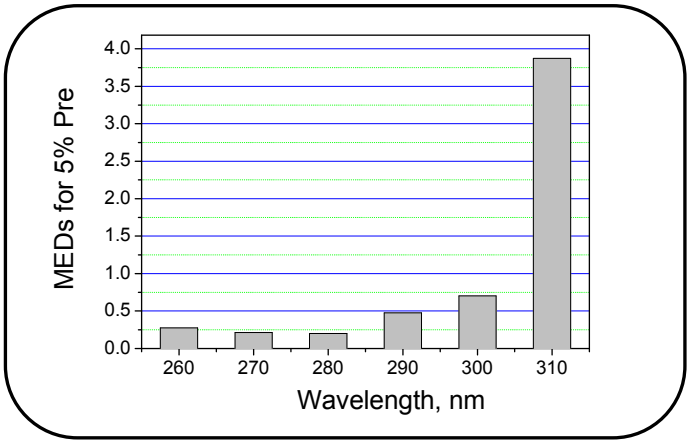


**Figure 13. The wavelength dependence of the MEDs quantity required to yield 5% of previtamin D.**

Additionally the action spectrum has been calculated for monochromatic irradiation ($\Delta\lambda$=1nm) within a spectral range of 250-320nm using the adequate system of differential equations which includes the term describing irreversible photoconversion Pre→Tox.. As may be inferred from Fig.14, rather good agreement between the experimental data and the calculated action spectrum is observed. A close relationship between the measured *in vitro* action spectrum of vitamin D synthesis and the absorption spectrum of provitamin D can be seen graphically.

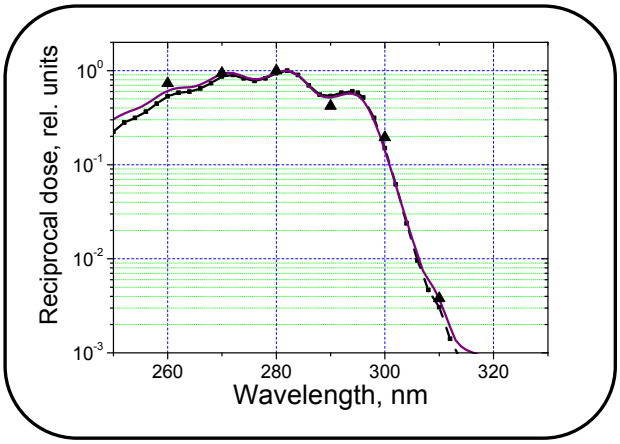


**Figure 14. The experimental data (symbols), the 7-DHC absorption spectrum (solid line) and calculated action spectrum of vitamin D synthesis (dash line).**

## 5 Critical review of the MacLaughlin et al paper (Science, 1982)

As mentioned above, action spectrum (AS) is defined as the spectral dependence of the value of the biological effect initiated by monochromatic radiation of different wavelength with the same dose.

A dose received after an exposure of $t$ : $E_{eff}\ t\ /\phi$ ,
where $\phi$ is numerical value in J/m$^2$ equivalent to one (antirachitic) quantity.

A correct action spectrum should retain both the quantitative **and** qualitative information of the data: the magnitude of the effect produced per photon and its wavelength distribution [Sutherland (1994)]. Moreover, a carefully constracted AS can identify the absorbing chromophore [Coohill, 1991].

The action spectrum of previtamin D3 formation from 7-DHC in human epidermis (Fig.15) shown in [MacLaughlin (1982)] was obtained by plotting the reciprocal of the dose as a function of wavelength. At any wavelength, no more than 5 percent of product was made.

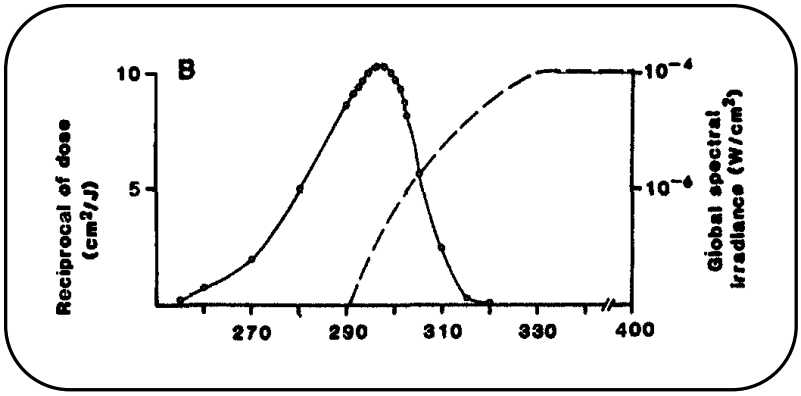


**Figure 15. Figure (B) from [MacLaughlin (1982)]: Action spectrum of previtamin D3 formation from 7-DHC in human epidermis (-o-) and spectral irradiance curve for sunlight (- - -)**

Now we need to examine next figure from the same reference [MacLaughlin (1982)] from the point of view of the role of the irreversible photodegradation. Fragment of the Figure 2 (A) from [MacLaughlin (1982)] shown in Fig.16, presents percent formation of PreD3 from 7-DHC in human epidermis (------) or from crystalline 7-DHC (10µg/ml) dissolved in tetrahydrofuran (- - -) after exposure to a range of doses of simulated solar radiation. If we compare this graph with Fig.10a,c) we can clearly see the establishment of photostationary state, i.e. the **concentration analysis did not take into account the irreversible photodegradation**!

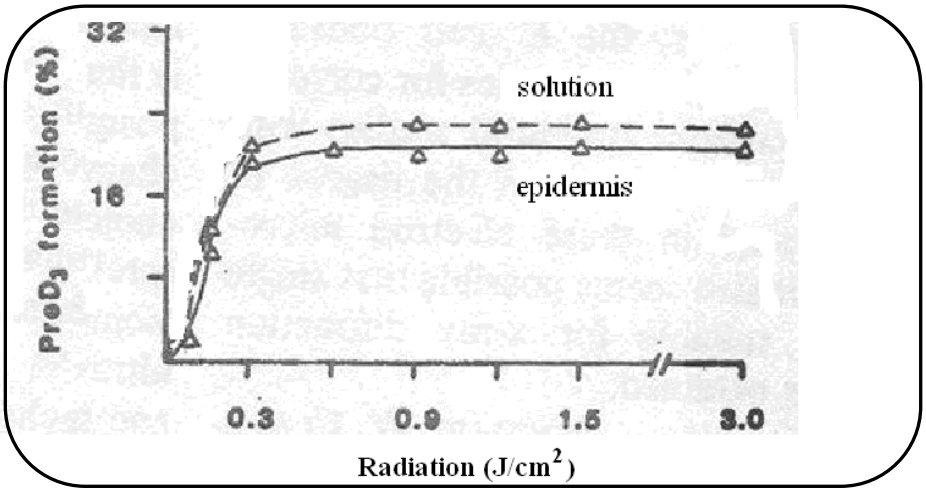


**Figure 16. Fragment of the Figure 2 (A) from [MacLaughlin (1982)]: percent formation of Pre from 7-DHC after exposure to simulated solar radiation measured by radiometer.**

Seeing very good coincidence of previtamin $D_3$ formation from 7-DHC in human epidermis (*in vivo*) and in solution (*in vitro*) in Fig.16 notwithstanding the difference between the two action spectra (in vivo and *in vitro*) in Fig.1, one can legitimately doubt that the action spectrum of PreD3 formation from 7-DHC in human epidermis is correct..

It seems plausible reason of such self-contradiction that concentrational analysis was fulfilled by MacLaughlin's *et al.* [MacLaughlin (1982)] within the limits of generally accepted photostationary approximation in provitamin D photochemistry assuming total concentration of the four main photoisomers equal to 100% with no regard for irreversible photoconversions of previtamin D. Really, the restrictions of the photostationary state approximation in provitamin D photochemistry was yet unknown in 1982, but as we showed in previous paragraph, such disregard introduced large errors into the measured concentrations.

## 6 Conclusion

UV irradiation of human skin [MacLaughlin (1982)] did not show any correlation of the action spectrum of previtamin $D_3$ photosynthesis with the absorption spectrum of provitamin D in spite of the fact that close coincidence of previtamin $D_3$ formation from 7-DHC was found in human epidermis and in solution.

We should consider at least **two reasons for the mismatch** between the *in vivo* and *in vitro* action spectra:

*i*) incorrect concentration analysis without proper consideration of the irreversible photodegradation (discussed above),

*ii*) so-called 'antenna effect' in skin similar to one described in [Nowakowska (1997)]. This can occur if the AS corresponds closely to the absorption spectrum of a molecule with the affected chromophore, which has an energy level configuration that matches the energy of the incident photons, but is not necessarily situated in the molecule that causes the ultimate effect. An energy transfer from the absorbing chromophore to the eventual target molecule can occur and can sometimes be measured [Coohill, 1991].

The possible energy scheme for the antenna effect is presented in Fig.17. It might be suggested that a molecule-chromophore (?) in the upper layer of skin can absorb the long-wave (UV-A) photons and transfer the excitation energy by non-radiative way to the $A_{2g}$ energy level of provitamin D from which conversion to previtamin D takes place. Direct optical excitation of this $A_{2g}$ energy level (located lower than the $B_{2u}$ one) is prohibited by symmetry.

Such scheme could explain how the photoconverion **Pro→Pre** could proceed without a UV-B photon absorption by provitamin D molecule. (additional study of the 'antenna effect' on previtamin D synthesis should take place).

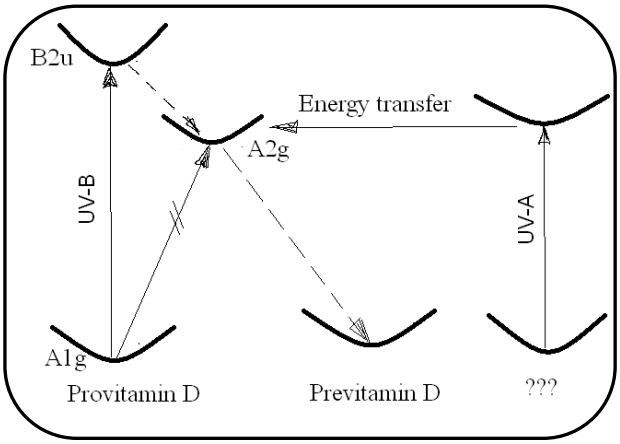


**Figure 17. Energy scheme explaining a possibility of previtamin D formation upon UV-A irradiation**

In conclusion it may be said that the revealed discrepancy between the *in vivo* and *in vitro* action spectra of vitamin D synthesis necessitates detailed consideration to avoid pitfalls in calculations of the vitamin-D-effective UV irradiance of the UV sources. This suggests the appropriateness of additional experimental work on simultaneous UV exposure of human skin and 7-dehydrocholesterol solution with correct concentration analysis.

Certainly, an ideal action spectrum would retain both the quantitative and qualitative information of the data: the magnitude of the effect produced per photon and its wavelength distribution. Just this quantitative aspect is lost in the CIE action spectrum for the production of previtamin D3 in human skin [Bouillon et al (2006)] which is based on McLaughlin's et al AS and presents only relative (unitless) response. This omission of the doses used at each wavelength causes the greatest criticism and raises doubts about the correctness of the AS in vivo [Norval et al, 2010]

It is clear that action spectrum analysis can be difficult and it is easy to obtain unexpected or even misleading results by incorrect treatments [Sutherland, 1994]. Therefore operating with quantitative data is needed to estimate the risk of getting sunburn in comparison with benefit for vitamin D synthesis.

In particular, Holick's rule [Dowdy et al (2010] which suggests that a full body exposure to 1 MED is the equivalent of an oral intake of 10000 IU (International Units) of Vitamin D3 does not have quantitative grounds, especially taking into account the differences in spectral dependence of the erythemal and 'antirachitic' action spectra. Note that later the conversion factors have been proposed that change erythemally weighted to previtamin D3-weighted UV doses [Pope et al. (2008)].

In the end it might be well to point out that the recently presented algorithm [Terenetskaya, Orlova, 2011] makes possible direct estimation of the vitamin D synthetic capacity of sunlight without using an AS. Moreover, the time dependence of production of previtamin D in vivo can be calculated by solving the system of rate equations with the spectrum of a UV source completed with UV transmittance of the skin [Meinhardt et al, (2009)] at the input [Terenetskaya (2011)].

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