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DEVELOPMENT OF PERSONAL UVB SENSOR: DETECTION OF PREVITAMIN D PHOTOSYNTHESIS

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INTRODUCTION

Although health-promoting properties of sunlight have been recognized from the beginning of civilization, predicted increase in solar UVB radiation (280-315nm) produced by ozone depletion has raised concerns on the resulting effects on human health and ecosystem. While there are both beneficial and harmful UVB effects, it is an increase in the harmful effects, which attracts most attention.

Excessive UV exposures are generally associated with acute and chronic health effects, such as erythema, skin cancer, immune system suppression, cataract. To raise public awareness and to provide information about potential risks of solar UV exposure, the use of a simple universally applicable solar UV index related to minimum erythema dose has been recommended by WHO in 1994 (1 MED = 200 J/m^2).

A number of personal UV dosimeters have been designed to measure ambient solar UV levels and give an indication of one's accumulated UV dose. At the moment variety of small electronic instruments are available on the market. For the most part the spectral sensitivity corresponds to the CIE erythema action spectrum [1], and anyone can easily determine exposure for healthy sunbathing according to the individual skin type.

Worthy of mention is passive chemical UV dosimeters that change optical characteristics when sunlight is applied to the materials. They have been developed for public use and calibrated for the erythemally effective exposure. The SUNTEST is a chemical UV dosimeter with silver-mercury-

oxalate suspension [2]. The accumulated UV dose can be evaluated *in situ* from the darkening of the dosimeter, from white to dark brown, comparing it with the calibration scale. Another chemical dosimeter - polysulphone film, has been used extensively for measurements of human exposure in epidemiological studies [3-5]. However, it is unable to provide UV dosimetry *in situ* because the laboratory analysis of an exposed material is needed with the use of UV absorption spectroscopy.

Furthermore, several biological dosimeters have been developed to indicate the effect of UV irradiance on a specific biological system over a designated period of time. They use biological material as a UV-sensitive target for assessing UV biological effectiveness, primarily its DNA-damaging capacity. But the result is (often) not available immediately after an exposure, as further (sometimes time-consuming) laboratory analysis of the dosimeter material is required to determine the degree of UV-initiated change. The examples are a Biofilm consisted of immobilized bacterial spores *Bacillus subtillis* [6,7], bacteriophage T7 in buffer solution [8] and solution of DNA molecule [9].

While excessive UV exposures are commonly associated with adverse health effects, small amounts of UV are beneficial for people and essential in the natural production of vitamin D_3 in skin. Natural exposure to sunlight is responsible for maintaining adequate vitamin D[1] nutrition for most of the population in the world, and the importance of sunshine as a therapeutic measure for preventing the bone deforming diseases is now appreciated. Moreover, in addition to the well-established effects of vitamin D on maintenance of mineral homeostasis, recent evidence has suggested a wider biologic role of vitamin D and its active metabolites in tissues not primarily related to mineral metabolism [10,11].

In particular, as reported by latest epidemiologic study [12], several types of cancer (breast, prostate, kidney, colon, ovary, and pancreas) have a strong association with low levels of D and lack of sunlight. It has been noted that northern countries have higher levels of cardiovascular disease and more heart attacks occur in winter months [13,14]. Vitamin D and/or sunlight (UV-B) have been shown to lower blood pressure, restore insulin sensitivity, lower cholesterol, and normalize food intake and blood sugar [15]. Low

[1] Two principal chemical species of vitamins - D_2, or ergocalciferol ($C_{28}H_{44}O$) and D_3, or cholecalciferol ($C_{27}H_{44}O$) are available. The terminology vitamin D is employed here in general sense. Both vitamins D_2 and D_3 are photochemically produced from their precursors ergosterol and 7-dehydrocholesterol (7-DHC). It is significant that basic monomolecular isomerizations of the two steroid species occur in perfect analogy.

vitamin D is associated with several autoimmune diseases including Multiple Sclerosis, rheumatoid arthritis, thyroiditis and Crohn's disease [15].

Hence it follows that UV dosimetry from the standpoint of its vitamin D synthetic capacity deserves particular attention, especially in the context of dramatic influence of seasonal and latitudinal changes in solar UVB radiation on the ability of sunlight to provide the human requirements for vitamin D [16].

UV DOSIMETRY USING AN *IN VITRO* MODEL OF VITAMIN D_3 SYNTHESIS

As is known vitamin D_3 is synthesized from 7-dehydrocholesterol through two consecutive monomolecular isomerizations catalyzed by UVB radiation. At the first step previtamin D is formed by photoinduced hexadiene ring opening. Further it is converted into vitamin D by thermoinduced intramolecular hydrogen shift. Consequently, concentration of accumulated previtamin D in a definite exposure time can serve as a measure of biologically effective 'antirachitic' UV dose. The problem is that side photoconversions of previtamin D give rise to multicomponent photoisomer mixture that drastically complicate the concentration analysis.

In the pioneering applications of an *in vitro* model of vitamin D_3 synthesis (ethanol solution of 7-dehydrocholesterol) the analysis was conducted using high performance liquid chromatography (HPLC) [17]. Obviously time consuming HPLC analysis is unable to provide the UV measurements *in situ*.

A major step toward an *in situ* dosimetry was made with development of original spectrophotometric analysis [18,19]. To measure the 'antirachitic' UV dose, the solution of 7-DHC in ethanol (C = 20 μg/ml) is exposed in rectangular quartz cuvette of 0.5÷1cm thickness, and UV absorption spectra of the solution are recorded within 230-330nm before and after an exposure to sunlight (or artificial UV light). Concentration of accumulated previtamin D is derived from the spectra by computer processing using specially designed program. This enables performance of the 'antirachitic' UV dosimetry *in situ* if portable spectrophotometer is available on site.

It is exceptionally important that adequate mathematical model has been developed which links biological and physical units and enables calculation of the photoreaction kinetics for any monochromatic or polychromatic UV

source with the intensity distribution $I_0(\lambda)$ [18]. It has been found that action spectrum of previtamin D photosynthesis (*in vitro*) calculated with the use of the model correlates well with the experimentally measured one.

Using a number of narrow band filters ($\Delta\lambda$ = 2 nm) and calibration facility at the Institute of Space Aeronomy (Brussels) the physical and erythemal doses required for accumulation of 5% previtamin D have been determined for different wavelengths [21] (Table 1). It is clear from the Table that the relation between physical, erythemal and 'antirachitic' doses is not constant with the wavelength, and because of this, may vary over a wide range with the constantly changing spectral composition of sunlight.

Table 1. Physical and erythemal doses required for accumulation of 5% of previtamin D under quasi-monochromatic UV irradiation at different wavelengths.

Wavelength (**nm**)	260	270	280	290	300	310
Previtamin D (**%**)	5	5	5	5	5	5
Erythemal dose (**MED**)	0.28	0.21	0.2	0.48	0.70	3.87
Physical dose (**J/m^2**)	55	42.7	40	95	205	10400

From the above discussion it appears that while the method is workable, it is not easily accessible in personal UV dosimetry in view of the use of liquid medium, UV spectrophotometer and computer processing of the absorption spectra.

Hence our task was to find a method for easier monitoring the previtamin D photosynthesis or even to find a possibility to make the process visible. With this aim we concentrated our attention on the nematic liquid crystals with intended use of provitamin D molecule as a chiral dopant [22].

It is believed that peculiar features of structure endow the molecule of 7-dehydrocholesterol with this ability. The molecule can be considered 'lath shaped' with dimensions approximately 4Å × 7Å × 20Å [23]. Actually, the molecule is not flat, but presents a corrugated appearance with a small twist about the long axis. In addition the methyl groups at carbons 10 and 13 protrude out of the molecular plane. By this is meant that the parallel layers

in nematic liquid crystal will be twisted with respect to one another by the action of 7-DHC dopant.

LIQUID CRYSTALLINE SENSOR

Basic features of nematic crystal with chiral dopant

Chiral dopant is optically active substance which induces cholesteric phase when added in small amount into nematic liquid crystal (LC). The director of the LC molecules adopts a helically twisted orientation under the action of chiral dopant (Fig.1).

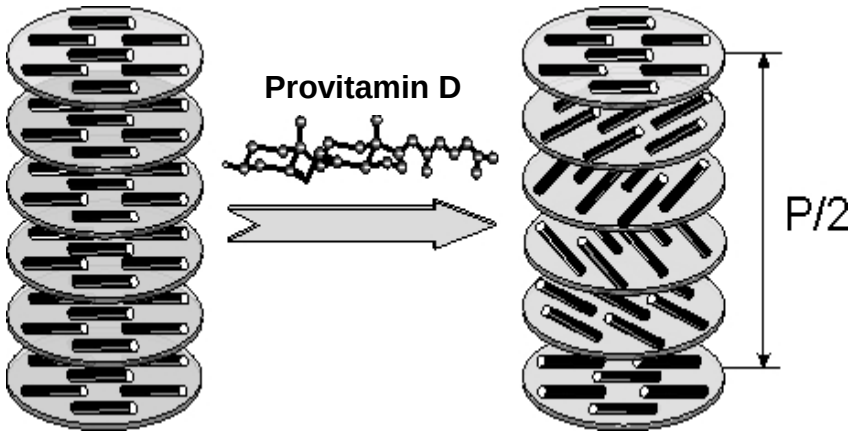

*Figure 1. Schematic representation of transformation of nematic LC into cholesteric phase induced by chiral dopant of provitamin D and an idealized representation of the order of a cholesteric liquid-crystalline phase. Each layer exhibits nematic-like order and the layers are twisted with respect to one another to form a macrohelix. The solute molecules are aligned such that their long axes lie parallel to the axis described by the solvent molecules. The distance it takes the director to complete a turn of 360^0 is called the cholesteric pitch **P** of the liquid crystal.*

If unpolarised white light is incident parallel to the helical axis, a narrow wavelength band will be reflected while all the others are transmitted. The critical wavelength λ_0 is determined by the helix pitch P and the average index of refraction n so that $\lambda_0 = P \cdot n$. This can introduce various colors of cholesteric LC if the wavelength of selectively reflected light λ_0 is in the visible region of the spectrum.

Since the molecular conformation of steroid moiety of provitamin D molecule is altered by the photochemical conversion into previtamin D, it is expected that cholesteric pitch and the wavelength for selective reflection will be affected and, as a result, the LC will vary in color. This could provide the easiest detection of previtamin D synthesis and evaluation of the accumulated UV dose *in situ* by comparison of the LC cell color with the calibration scale.

To fulfill the design requirements for host matrix, the LC should be transparent in the UVB range, thermally stable over the temperature interval at least 10-40^{0}C, be a good solvent for 7-DHC and be stable in relation to visible light. Two nematic liquid crystals LC-805 (NIOPIK, Russia) and ZLI-1695 (Merck) have been selected as host matrices, and 7-DHC (Sigma) was dissolved in the LC material in concentration 5÷10 wt.%.

Although on dissolving 7-DHC in the nematic LC the appearance of a papillary texture characteristic for induced cholesteric phase has been observed with a polarizing microscope, however we have not detected any coloration of the LC up to the 7-DHC's solubility limit. Thus, it was concluded that the 7-DHC molecule possessed inadequate helical twisting power (HTP)[2] to ensure required helix pitch.

Fortunately, it occurred to us that this difficulty could be obviated by use of a wedge-like cell. As is known a chiral nematic (cholesteric) LC, filled into a cell which was treated to provide a homogeneous alignment, forms a Grandjean texture [24] in which quasi-nematic layers are twisted (see Fig.1), and over the distance corresponding to the cholesteric pitch the director would perform a complete turn.

If the walls of the cell are not parallel to each other, but form a wedge, the width of the cell is proportional to the distance from the origin of the wedge angle. At certain distances the spacing between the cell surfaces equals multiple values of half of the natural pitch of the cholesteric helix (P/2) (see

[2]At the dopant concentrations $C < 10$ % (by the mass fraction) the pitch is inversely proportional to C, and the constant of proportionality is called the helical twisting power (HTP) of the chiral dopant. The HTP is defined as: $HTP = (P \cdot C)^{-1}$

Fig.2). If the LC molecules are oriented parallel at both surfaces of the cell, the helix is distorted between these positions. With increasing cell spacing the cholesteric helix is at first elongated until a new half turn of the director is added and a compressed helix with an additional half turn is formed. This change in total twist leads to the formation of disclination lines (Cano) [24]. The disclinations occurring at equal distances from one another can be easily observed with a polarizing microscope.

EXPERIMENTAL

The wedge-like cell was prepared using Mylar spacer between two quartz plates (15 mm × 20 mm) as substrates. The cells thickness was measured by interferometer ($\boldsymbol{L}$ = 63μm). To provide planar boundary conditions the quartz substrates were spin coated with polydimethylsiloxane annealed on the hot plate at 200-250^{0}C over 4 hours and mechanically rubbed to ensure planar uniform alignment of the LCs. To avoid oxidation of 7-DHC the LC cells were carefully stuck along the perimeter. With these cells it was possible to follow the photoreaction course of provitamin D photoisomerization by recording the UV absorption spectra and, in parallel, by visual observations of the cells using polarizing microscope.

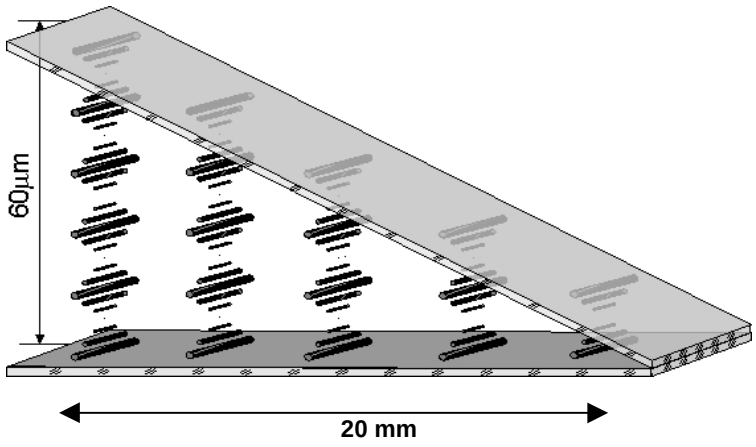

Figure 2. Schematic representation of the wedge-like cell and formation of disclination lines (Cano) in chiral nematic (cholesteric) liquid crystal.

Transformation of the nematic LCs into cholesteric phase was evidenced by observation the Cano lines when the cell was sandwiched between crossed polarizers. Well known dependence of the number of Cano disclinations ($\boldsymbol{N}_C$) on the dopant concentration ($\boldsymbol{C}$) has been detected. $\boldsymbol{N}_C$ =10 has been found at $\boldsymbol{C}$ =10 wt.% that corresponded to maximum solubility of 7-DHC. The cholesteric pitch $\boldsymbol{P}$ was calculated by equation $\boldsymbol{P} = 2\boldsymbol{L}/\boldsymbol{N}_C$ [24], and the changes of $\boldsymbol{P}$ were detected within the range of 9 ÷ 24 μm depending on the concentration of 7-DHC. What this means is that the selective reflection wavelength lies outside the visible spectrum (as it has been suggested earlier).

UV lamp irradiation

The sample with initial number of the Cano lines $\boldsymbol{N}_C$ =5 was irradiated with fluorescent lamp (EL-30) of integral UV intensity 0.5 mW/cm^2 within spectral range 260-350nm (Fig.3).

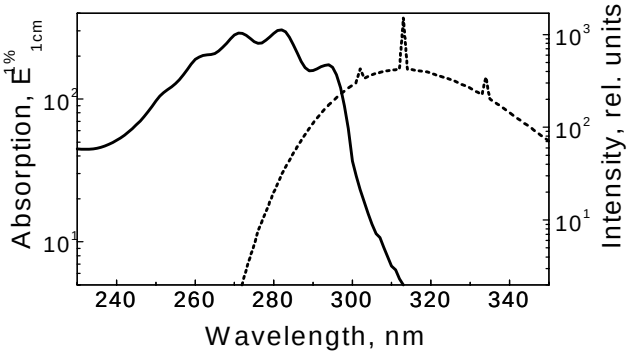

Figure 3. The EL-30 lamp radiation spectrum (dash line) and the absorption spectrum of Provitamin D_3 (solid line).

The decrease in the N_c with increasing irradiation time has been detected by placing the sample between crossed polarizers (Fig.4). This indicates that 7-DHC undergoes photoinduced transformation into the photoisomer possessing considerably lesser helical twisting power (HTP). Obviously, HTP of 7-DHC molecule with its rigid steroid skeleton may be significantly decreased by hexadiene ring-opening into conformationally flexible molecule of previtamin D. This gives rise to the increase in the cholesteric pitch and, as a result, is accompanied by the N_c decrease.

Consequently, by this means our findings hold promise for dosimetry of biologically active UVB solar/artificial radiation (280 – 315nm) by visual observation the Cano lines decrease with UV dose. This distinctly simplifies previously suggested method of UV dosimetry based on the spectral monitoring previtamin D photosynthesis in ethanol solution [19,20].

a) b) c)

Figure 4. Photographs of the LC wedge cell sandwiched between crossed polarizers that are illustrative of the Cano lines decline as a result of the UV lamp irradiation: a) $N_C = 5$ at $t = 0$; b) $N_C = 4$ at $t = 25$min; c) $N_C = 3$ at $t = 60$min.

Solar irradiation

It is important for the UV biodosimetry to have great confidence that the decrease in the Cano stripes number in the LC cell is caused by previtamin D formation. To be certain that the N_C decrease closely corresponds to an *in vitro* previtamin D photosynthesis the LC cell (ZLI-1695 with 7-DHC dopant) and the cuvette with ethanol solution of 7-DHC (C = 20μg/ml) were simultaneously exposed to sunlight around noon (clear day).

Before the exposure the UV absorption spectra of both samples had been recorded by the spectrophotometer KSVU-23 (LOMO, Russia), and the

initial number of the Cano lines in the LC cell had been fixed (N_C = 10 at t = 0). In the course of solar irradiation the number of the Cano lines in the LC cell was checked time by time by placing the cell between crossed polarizers. At the moment when one Cano line being disappeared the absorption spectra of both samples had been recorded again. The same procedure was repeated to the total exposure time of 200 min. During the night the samples were stored at the refrigerator, and on the next day were exposed 160 min in total until Cano lines number reduced to N_C = 5.

To derive the concentration of accumulated previtamin D the absorption spectra of the solution were processed with computer according to the original program in which known individual spectra of the photoisomers were used as the reference ones [19]. However, spectral analysis of the LC samples was impeded because of small shift and distortion of the 7-DHC spectrum in the LC matrix. Nevertheless, HPLC analyses of 7-DHC photoisomerization in LC [25] and in polymer film [26] showed that the same photoproducts were formed as in the solution.

Accumulation of previtamin D in ethanol solution during the exposure to sunlight and the associated changes in the Cano lines number in the simultaneously exposed LC cell are presented in Fig. 5.

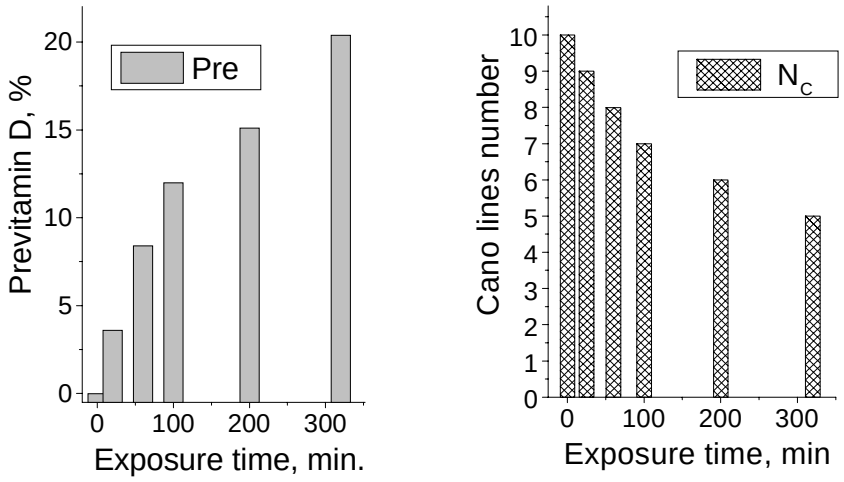

Figure 5. Accumulation of previtamin D in ethanol solution (left) and decrease in the Cano lines number in the LC cell during simultaneous exposure to sunlight
(August 21, 2000, local time 12.00-15.20 and August 22, 2000, local time 12.40-15.00)

From the result obtained it may be deduced that dependence of the Cano lines number on the accumulated UV dose proceeds in close agreement with photosynthesis of previtamin D in solution. This conclusion is more convincingly demonstrated in Fig.6, where one can see wholly satisfactory linear relationship between the N_C and previtamin D_3 concentration. From the data presented in Fig.6 one can find that $\Delta N_C = -1$ corresponds to ~4% concentration of previtamin D, that is ~0,8 μg/ml of previtamin D accumulation *in vitro*.

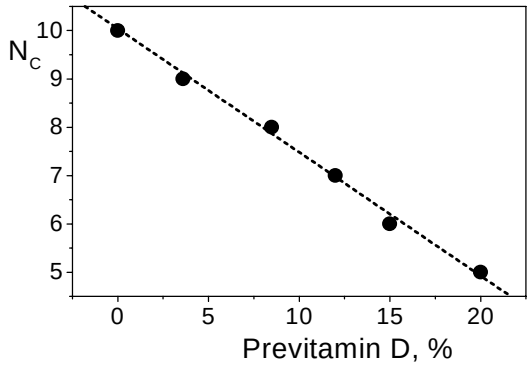

Figure 6. Correlation between the Cano lines number N_C in the LC cell and Previtamin D_3 accumulation in ethanol solution.

CONCLUSIONS

A new biosensor of vitamin D synthetic capacity of solar/artificial UVB radiation has been developed that is based on nematic liquid crystal with provitamin D_3 dopant. For the first time it has been experimentally demonstrated that nematic liquid crystals (LC-805, ZLI-1695) are converted into induced cholesteric phase by doping with chiral steroid biomolecules of provitamin D_3.

During UV exposure of the admixed LC in a wedge-like cell the decrease in the Cano lines number has been observed as a result of UV initiated photoisomerization which changed helical twisting power of the dopant molecules of 7-DHC. Excellent correlation between this clearly defined N_C dependence and previtamin D accumulation *in vitro* has been established. This suggests that the decrease in the N_C on the exposure time is well representative of the accumulated dose of biologically active "antirachitic" UV radiation, and after calibration the sensor can be used as personal UV-B biodosimeter.

In this manner the doped LC film can ensure personal monitoring the vitamin D synthetic capacity of sunlight by visual observation with crossed polarizers. The sensor great advantage is that it can be used to evaluate UV exposure in realistic conditions of changing orientation and surface position, e.g. for investigating exposure of different body parts during various activities. Further attempts will be directed to greater simplification of the procedure by shifting the selective reflection band into visible spectral region.

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REFERENCES

1. Commission Internationale d'Eclairage. A reference action spectrum for ultraviolet induced erythema in human skin. C.I.E. J. 1987; 6:17-22.
2. Horkay I, Wikonkal N, Patko J, Bazsa G, Beck M, Ferenczi A, Nagy Z, Racz M, Szalay T. SUNTEST: a chemical UVB radiation dosimeter. J. Photochem. Photobiol. B:Biol. 1995; 31:79-82.
3. Davis A, Deane GHW, Diffey BI. Possible dosimeter for ultraviolet radiation. Nature 1976; 261:169-179.
4. Airey DK, Wong JCF, Fleming RA, Meldrum IR. An estimate of the total UVB exposures for outdoor workers during a south-east Queensland summer. Health Phys. 1997; 72(4):544-549.
5. Krins A, Dorschel B, Henniger J, Knuschke P, Bais A. Readings of polusulphone film after fractionated and continuous exposures to UV radiation and consequences for the calculation of the reading resulting from polychromatic UV radiation. Radiat. Prot. Dosim. 1999; 83:303-308.

6. Quintern LE, Puskeppeleit M, Rainer P, Weber S, ElNaggar S, Eschweiler U, Horneck G. Continuous dosimetry of the biologically harmful UV radiation in Antarctica with the biofilm technique. J. Photochem. Photobiol. B:Biol. 1994; 22:59-66.
7. Horneck G. Quantification of biological effectiveness of environmental UV radiation. J. Photochem. Photobiol. B: Biol. 1995; 31:43-49.
8. Ronto G, Gaspar P, Berces A. Phages T7 in biological UV dose measurement. J. Photochem. Photobiol. B: Biol. 1992; 12:285-294.
9. Regan JD, Yoshida H. DNA UVB dosimeters. J. Photochem. Photobiol. B: Biol.1995; 31:57-61.
10. Mayar Anita, Norman Anthony. "Vitamin D", in*: Encyclopedia of Human Biology*, Academic Press, Inc. 1991; Vol. **7:**859-871.
11. Reichel H, Koeffler HP, Norman AW. The role of the vitamin D endocryne system in health and disease. N. Engl. J. Med. 1989; 320:980-991.
12. Grant WB. An estimation of excess cancer mortality in the United States due to inadequate doses of solar ultraviolet-B radiation and/or vitamin D.
13. Segall JJ. Latitude and ischemic heart disease (letter). Lancet 1989; 1:1146.
14. Williams FL, Lloyd OL. Latitude and heart disease (letter). Lancet 1989; 1:1072-1073.
15. Sullivan K. Preliminary Report on the importance of sunlight and vitamin D. http://www.sunlightandvitamind.com
16. Webb AR, Kline LW, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 in human skin. J. Clin. Endocrinol. Metabol. 1988; 67:373-378.
17. McLaughlin JA, Anderson RR, Holick MF. Spectral character of sunlight modulates photosynthesis of previtamin D3 and its photoisomers in human skin. Science 1982; 216:1001-1003.
18. Terenetskaya IP. Provitamin D photoisomerization as possible UVB monitor: kinetic study using tunable dye laser. *SPIE* 1994; 2134B:135-140.
19. Terenetskaya IP. Spectral monitoring of biologically active solar UVB radiation using an i*n vitro* model of vitamin D synthesis. Talanta 2000; 53:195-203.
20. Galkin ON, Terenetskaya IP. 'Vitamin D' biodosimeter: basic characteristics and potential applications. J. Photochem. Photobiol. B:Biology 1999; 53:12-19.
21. Bolsée D, Webb AR, Gillotay D, Dörschel B, Knuschke P, Krins A, Terenetskaya I. Laboratory facilities and recommendations for the characterization of biological ultraviolet dosimeters. Applied Optics 2000; 39: 2813-2822.
22. Leder LB. Rotatory sense and pitch of cholesteric liquid crystals. J. Chem. Phys. 1971; 55:2649-2657.
23. Carlisle CH, Crowfoot D. Proc. Roy. Soc. (London) 1945; A184:64-69.
24. De Gennes, Paul, *The Physics of Liquid Crystals*, Oxford: Clarindon Press, 1974.
25. Cassis EG, Weiss RG. Liquid-crystalline solvents as mechanistic probes--V. An investigation of the effect of cholesteric order on the formation rates of vitamin

D_3 from pre-vitamin D_3 and of pre-vitamin D_3 from vitamin D_3. Photochem.&Photobiol. 1982; 35:439-444.
26. Majima T, Nakamura Y, Sato T. Waseda University Bulletin 1991; 13:13-15.