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Long-Term Dosimetry of Solar UV Radiation in Antarctica with Spores of *Bacillus subtilis*

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The main objective was to assess the influence of the seasonal stratospheric ozone depletion on the UV climate in Antarctica by using a biological test system. This method is based on the UV sensitivity of a DNA repair-deficient strain of *Bacillus subtilis* (TKJ 6321). In our field experiment, dried layers of *B. subtilis* spores on quartz discs were exposed in different seasons in an exposure box open to solar radiation at the German Antarctic Georg von Neumayer Station (70°37'S, 8°22'W). The UV-induced loss of the colony-forming ability was chosen as the biological end point and taken as a measure for the absorbed biologically harmful UV radiation. Inactivation constants were calculated from the resulting dose-response curves. The results of field experiments performed in different seasons indicate a strongly season-dependent trend of the daily UV-B level. Exposures performed at extremely depleted ozone concentrations (October 1990) gave higher biologically harmful UV-B levels than expected from the calculated season-dependent trend, which was determined at normal ozone values. These values were similar to values which were measured during the Antarctic summer, indicating that the depleted ozone column thickness has an extreme influence on the biologically harmful UV climate on ground.

Springtime ozone depletion over Antarctica has been observed for approximately 10 years (4). In contrast to the numerous satellite and ground-based ozone measurements performed in Antarctica, determinations of the biologically harmful UV-B radiation are rare (UV-B, λ = 280 to 315 nm). Since the absorbing ozone layer influences the UV-B irradiance but not the UV-A on ground, these UV measurements are important to assess a possible influence of a changed UV climate on the biosphere. The first spectroradiometric measurements with a double monochromator were done just a few years ago (9). This instrument measures the physical energy of radiation incidence for the individual wavelengths within the UV region. But the biological effectiveness of the different wavelengths varies within this region. For example, the wavelength λ = 300 nm has a biological effectiveness approximately 100 times higher than that of the wavelength λ = 320 nm. This is the case for several biological end points such as mutation induction and cell inactivation. To determine the biological effect of UV radiation in terms of the effective UV dose on the basis of physical data, a calculation is necessary. The convolution of an appropriate action spectrum with the solar irradiance spectrum with its cutoff for short wavelengths at approximately 290 nm may be a convenient method. A disadvantage of this method is the fact that polychromatic UV radiation (solar spectrum) has been shown to produce biological effects that may differ by up to an order of magnitude from those of monochromatic radiation (6). Since most action spectra are based on monochromatic radiation studies, the above-mentioned method of computing the irradiance spectrum with an action spectrum as a correction function may lead to artificial results. Despite these problems, physical UV data from Antarctica were computed by using the action spectrum for DNA damage (13), and the calculated biologically effective UV dose was compared with meteorological and ozone data (15).

A biological system, directly weighting the radiation by its biological efficiency, seems to be a practical alternative to the method mentioned above. Using bacterial spores as an integrating UV detector, the wavelengths will be weighted by their cytotoxic effects without the complicated and expensive setup of instruments necessary for physical dosimetry (10, 11, 17, 18). Despite the fact that many biological systems differ in their action spectrum for radiation-induced damage since they contain different chromophores, they have in common the most important chromophore for UV-induced damages—the DNA. As a consequence, many biological systems offer a similar action spectrum within the UV-B region, a spectral region in which DNA has a high absorption coefficient. The initial damage leading to cell death, mutation, and carcinogenesis is believed to occur in the DNA. The action spectrum for spore inactivation also resembles the action spectrum of other systems in the UV-B region (13). Therefore, the rate of UV-induced spore inactivation may be used as a measure for biologically effective radiation in other biological systems also. With vegetative cells of a mutant strain of *Escherichia coli* as the UV dosimeter, measurements were possible even within the water column of the Antarctic sea (8).

Spores of *Bacillus subtilis* have been tested as a biological dosimetric system suitable for monitoring solar UV radiation

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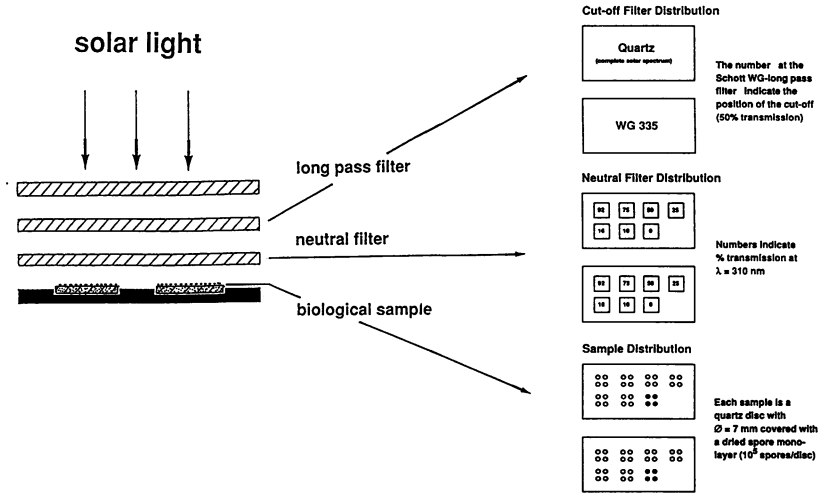

FIG. 1. Schematical drawing of the optical system of the exposure facility and of the sample disc and filter distribution.

by different investigators (10, 11, 17). Therefore, this well-characterized microorganism was chosen to realize the first long-term biological UV dosimetry in Antarctica. As a first step to continuous long-term UV dosimetry, we exposed dried spores of *B. subtilis* to solar radiation on sunny days in different seasons. The inactivation constants determined were compared with the solar elevation angle at noon at the exposure day and with the ozone column thickness above Georg von Neumayer Station (data from Total Ozone Mapping System [TOMS] of the NASA satellite Nimbus 7).

MATERIALS AND METHODS

Bacterial strains and sample disc preparation. Spores of *B. subtilis* TKJ 6321 (*uvrA10 ssp-1 polA151 hisH101 metB101* [16]) defective in DNA excision repair and a spore-specific DNA repair mechanism were kindly provided by N. Munakata. Spore stocks were prepared in sporulation medium and purified as described before (12). Dry monolayers of spores on quartz discs (10^5 spores per disc) were prepared as follows. Quartz discs (7-mm diameter) were silanized in a solution of 5% (vol/vol) dimethyl-dichlor-silan in $CHCl_3$ for 20 min and were subsequently washed with $CHCl_3$, ethanol, and finally with water. Then the inner zone of each disc (5-mm diameter) was again desilanized by exposure to a Bunsen burner flame for 4 s, during which the rim of each disc was protected by a metal mask. By this method, a hydrophilic circular surface surrounded by a hydrophobic rim was obtained. An aqueous spore suspension (20 μl) was spread on the hydrophilic area of each disc and dried in laboratory air. The hydrophobic rim of each sample disc was maintained spore free to avoid shadowing by the collimator used during the exposure (5). Approximately 1,000 sample discs were prepared in Cologne, Germany, in October 1989. They were stored in pine wood trays and kept in the dark at ambient temperature. Similar trays and conditions were utilized for shipment back to Germany after the exposure experiments, where the biological analysis was performed at the DLR. Unexposed samples were used as controls.

Sunlight exposure. The sample discs were mounted on two black exposure trays (each accommodating 28 samples) inside an exposure box. They were exposed beneath a filtering system composed of neutral-density filters (transmission rates of 10, 16, 25, 50, 75, and 92%), long-pass filters (quartz, 2 mm, with a high transmission rate for the whole solar spectrum, and WG 335, 2 mm [Schott, Mainz, Germany], with 50% transmission at 335 nm), and a quartz cover for weather protection (Fig. 1). Four parallel samples were mounted beneath each filter combination; dark controls were also available. Temperature was kept at 25°C by use of an active temperature control. In addition, an Si detector beneath a bandpass filter for $\lambda = 350 \pm 4$ nm was integrated in the exposure box. The relative values of the measured fluence rates were electronically integrated, giving a relative value of the UV-A dose during the whole exposure experiment. The exposure box was mounted on a tripod and positioned on the shelf ice at the German Antarctic Georg von Neumayer Station (70°37'S, 8°22'W). The biological UV dosimetry campaign lasted from February 1990 to January 1991. Exposures were done preferably on sunny days, starting at local noon and lasting until late afternoon or until noon of the next day. The appropriate exposure times were derived from the ozone-independent relative UV-A doses given by the Si detector (the doses required were determined beforehand in pilot experiments). Every 30 min, the orientation of the box was adjusted to the vertical incidence of solar radiation. Each data set provided two dose-response curves, one for the whole solar spectrum and one for the spectral range transmitted by the WG 335 filter (transmission spectra in Fig. 2). In the latter case, solar UV-B radiation was omitted.

Data evaluation. After transport back to Germany, the

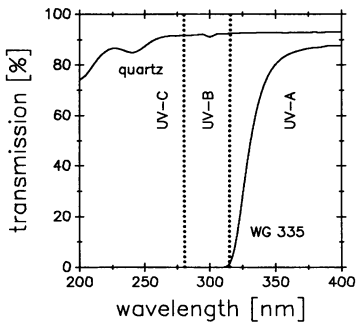

FIG. 2. Transmission curves of quartz and WG 335 filters.

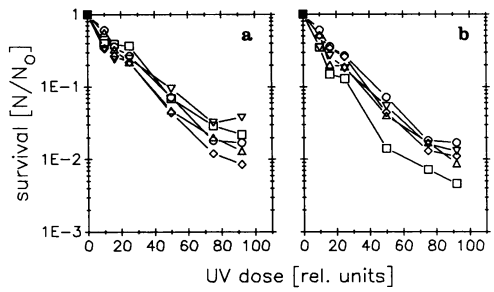

FIG. 3. Inactivation curves of samples with different storage times before irradiation (a) and after irradiation (b). Symbols for storage times (months): ○, 0; △, 1; ◇, 2; □, 6; ▽, 12.

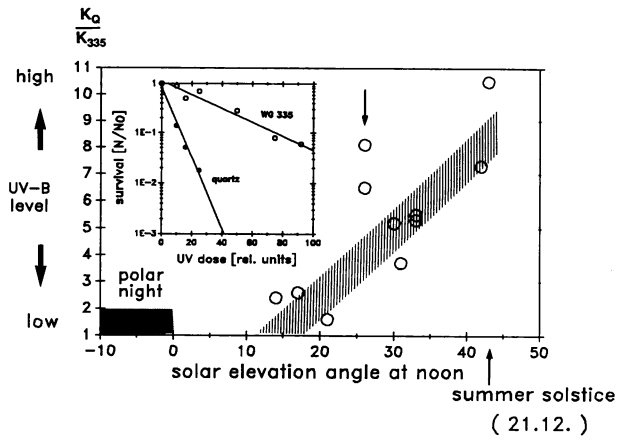

FIG. 4. Ratio of inactivation constants determined behind quartz (K_Q) divided by the inactivation constant determined behind the long-pass filter WG 335 (K_{335}) as dependent on the solar elevation angle at noon. The linear regression line was calculated without the two values determined under the ozone hole, which are marked with an arrow (compare with Table 1). These two points belong to the linear regression line with a confidence level of <5%, indicating that these values do not fit the season-dependent trend. The insert shows the data from a representative experiment giving the inactivation curves for a solar elevation angle of 33 degrees and 288 DU, determined under quartz and under a WG 335 filter.

spores were recovered from the discs and suspended in 1 ml of water as described before (1). Different dilutions were plated on agar plates (8 g of nutrient broth and 4 g of NaCl per liter) by using a spiral plater (Spiral Systems Inc., Cincinnati, Ohio). Each value was determined at least in duplicate. After incubation at 37°C for 16 h, colonies were counted and the inactivation constants were determined by using the equation [In (N/N_0)]/fluence = $-K$, with N representing the number of colony formers per irradiated sample disc, N_0 representing the number of colony formers of the control, fluence showing the fluence given in relative values deduced from the transmission rate of the used neutral density filter, and $-K$ being the inactivation constant.

For each of the two data sets, the inactivation constants were calculated (complete spectrum [quartz] and spectrum without UV-B [WG 335]). The quotient of K_{quartz} (K_Q) divided by K_{335} was taken as a measure for the biologically effective UV-B level at the exposure day. High values represent a high UV-B level (a value of 1 is the minimum for solar radiation at identical inactivation constants for the complete spectrum and for the spectrum filtered through a WG 335 filter). The resulting UV-B levels were compared with the solar elevation angle at noon and with the ozone column thickness above Georg von Neumayer Station at the exposure day. Ozone data are from TOMS aboard the NASA satellite Nimbus 7. Values are given in dobson units (DU). A value of 100 DU represents an ozone layer thickness of 1 mm at 1 atm (101.3 kPa) and 298 K.

Control for spore UV sensitivity after different storage conditions. Because of experimental constraints, sample discs with a different history of storage before and after exposure had to be used. To investigate the influence of these conditions, a set of sample discs was prepared at $t = 0$ and exposed, while covered with a set of neutral density filters, to an artificial UV-light source (solar light simulator SOL-2; Dr. Hönle, Martinsried, Germany) after different storage times (0, 1, 2, 6, and 12 months). The survival curves were prepared immediately after irradiation. To test a possible effect of different storage times performed after UV exposure, we irradiated several sample disc sets at the same day but with the discs covered with different neutral-density filters. The survival curves were determined after different storage times (0, 1, 2, 6, and 12 months).

RESULTS

In addition to the long-term UV dosimetry in Antarctica, the influence of different storage times before and after spore irradiation was tested. Figure 3 shows the resulting survival curves in a semilogarithmic manner. No influence of the storage time (storage before or after spore irradiation) could be detected, and no spore inactivation could be detected with the dark controls after a storage time of 1 year (data not shown).

Field exposures were done at Georg von Neumayer Station before the polar night from 15 February 1990 to 6 April 1990 and after the polar night from 7 October 1990 to 15 January 1991 (test exposures in January 1990). Despite bad weather conditions such as low temperatures, storms, and snow, a total of 11 exposure experiments were successfully completed. A representative example of the two inactivation curves that were obtained on one exposure day (15 February 1990) is shown in Fig. 4. Measurements were spread over the year, at different seasons, with sun elevation angles at noon ranging from 14 degrees up to 43 degrees. Table 1 gives the solar elevation angle at noon, the ozone data, and the inactivation constants K_Q and K_{335}, determined for each exposure day, as well as the ratio of K_Q and K_{335}. The latter value is a measure for the biologically effective UV-B level. In Fig. 4, K_Q/K_{335} is plotted as a function of the solar elevation angle at noon. Data that were obtained at the normal ozone column thickness of 252 to 332 DU indicate a season-dependent trend of the UV-B level, which increased with solar elevation angle and reached the highest values during summer (January).

Two exposure experiments were performed at highly reduced ozone values on 7 and 8 October 1990 (compare with Fig. 5). The UV-B levels of these experiments do not fit into the season-dependent trend deduced from the other exposure experiments at normal ozone values. At ozone values of about 160 DU, the UV-B levels are about twice as high as expected at the same solar elevation angle (26 degrees) from the season-dependent trend (Fig. 4). In this period of the ozone hole, the UV-B level reaches values that are normal during the Antarctic summer.

TABLE 1. Biological UV dosimetry at Georg von Neumayer Station, Antarctica

Solar elevation angle at noon (degrees)	Date	Ozone (DU)[a]	K_Q/K_{335}	K_Q	K_{335}	Exposure time (h)
14	6 April 1990	258	2.4	−0.043	−0.018[b]	6
16	31 March 1990	270	2.6	−0.037	−0.014	20
21	19 March 1990	252	1.6	−0.028	−0.018	23
26	7 October 1990	158	8.1	−0.145	−0.018[b]	21
26	8 October 1990	174	6.5	−0.129	−0.02	26
30	24 February 1990	304	5.2	−0.094	−0.018	3.5
31	23 February 1990	318	3.7	−0.086	−0.023	2.5
33	15 February 1990	288	5.5	−0.16	−0.029	27
33	17 February 1990	288	5.3	−0.064	−0.012	3
42	15 January 1991	293	7.3	−0.154	−0.02	4
43	4 January 1991	332	10.5	−0.189	−0.018[b]	3

[a] Data from TOMS aboard the NASA satellite Nimbus 7.
[b] Average value for K_{335} (n = 12).

DISCUSSION

The season-dependent reduction of stratospheric ozone over Antarctica during recent years led to a changed UV climate in Antarctica which was demonstrated by spectroradiometric measurements (9). The increased UV-B levels during the austral spring may have consequences on the biosphere (3, 7, 14). Antarctic marine and terrestrial ecosystems may be influenced by increased UV-B stress with yet unknown consequences. To answer this question, continuous dosimetry of the biologically harmful radiation is necessary. As a first step in our first experiment, monolayers of dried bacterial spores were used as the bioindicator. This system was used in combination with a filter system to discriminate between ozone-independent UV-A and ozone-dependent UV-B effects. The data in Fig. 4 and Table 1 indicate that the bacterial system we used has a sensitivity profile which enables the detection of ozone-dependent changes of biologically effective UV-B within the solar spectrum. An ozone- and UV-B-dependent response was also demonstrated in Antarctica with a mutant strain of *E. coli* within the water column (8). This was possible because the action spectrum for UV-induced cell damage in *E. coli* is similar to the action spectrum for the inactivation of *B. subtilis* spores. In contrast to the complex exposure facility used in our experiment, the *E. coli* exposure system was simple, which made exposure within the water column possible.

The 11 exposure experiments (Fig. 4), integrating the radiation effect of up to 1 day, were performed from February 1990 to January 1991. This enabled only a fragmented documentation of the ozone- and season-dependent UV-B level. Thus, the results obtained from our experiment are deduced from a discontinuous data evaluation and should be controlled by using the same or a similar dosimetric experiment in Antarctica. A remarkable similarity exists between our result (an increase of UV-B during the ozone depletion period in October up to values typical for the summer) and calculated values for the biologically effective UV dose deduced from spectroradiometric measurements performed at McMurdo Station, Antarctica, in 1990 (2). On the one hand, this indicates the practicability of the theoretical determination of biological effective doses on the basis of physical data, and on the other hand, it indicates the applicability of a biological detection system as a biosensor for the detection of ozone-dependent changes in the UV climate on Earth. Based on the data presented here obtained with *B. subtilis* spores, a calculation of the possible response of other organisms exposed to similar radiation conditions must be done with care. Different factors may lead to different action spectra and sensitivities in other biological systems. In contrast to *B. subtilis*, many other organisms perform photoreactivation to eliminate DNA damage, and other photoproducts are induced since other organisms lack small acid-soluble spore proteins and have DNA repair systems with different efficiencies compared with *B. subtilis* spores. In addition to these biochemical factors, environmental conditions and adaptive mechanisms may lead to different UV-B sensitivities (14). Despite these differences, many systems offer a similar action spectrum within the UV-B region (13) since DNA is one of the main targets for UV-induced damage.

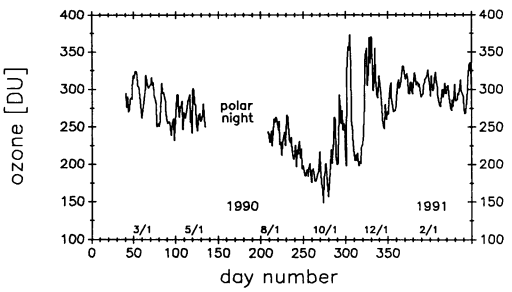

FIG. 5. Total ozone during the time period 1990 to 1991 above Georg von Neumayer Station. Data were taken from TOMS, NASA, Nimbus 7.

Besides the high sensitivity of the biological test system used here, the method has disadvantages, such as the time-consuming procedure of moving the exposure facility so it is always in a suitable orientation toward the sun and the time-consuming method necessary for determining the spore survival curves and data calculations. Aiming to improve the method, a modified system for biological UV dosimetry, a biofilm with *B. subtilis* spores immobilized on a transparent polyester sheet, was developed (12). The new exposure facility containing the biofilm is now being used in a second long-term dosimetry experiment in Antarctica. To obtain a detailed data base for the changed UV climate at reduced ozone concentrations with its possible consequences on the biosphere, an international network of stations using well-calibrated standardized biological UV do-

simetry will be necessary, and the data should be compared with the ozone values, meteorological data, and biologically effective dose calculations deduced from spectroradiometric measurements.

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