pared specimens and the ethanol-stored samples. Although the minimum soaking time required to sufficiently soften the material varied between samples (presumably a function of their original collection/storage regime), there appeared to be no risk of over softening the tissue and thus losing delicate surface features. Only in six samples did saponin fail to soften the organs sufficiently to evert them. In these instances the tissue had been left in the solution for 2.5 h and appeared to have regained sufficient flexibility for eversion. However, upon attempting eversion we found that the material crumbled, as the tissue inside was still hard and brittle. Longer soaking times may have been required for these samples, or some samples simply may not be reclaimable using this method.

The use of fixed material to investigate the surface features of squamate hemipenes may always be, at best, a salvage operation. This is often necessary because of the lack of suitably preserved specimens. We found that saponin is a highly effective softening agent of formalin-fixed material, as not only are preparation times relatively short, but the treatment is also very mild to the tissue. Consequently delicate structures on the surface of the organ are retained throughout processing. It is possible that other solutions with detergent-like properties may prove successful in salvaging similarly preserved tissues.

*Acknowledgments.*—We are very grateful to R. Coory for permission to dissect preserved specimens from the Museum of New Zealand Te Papa Tongarewa, and T. Whitaker and B. Thomas for the provision of ethanol-stored specimens of *Hoplodactylus maculatus*. We also thank J. Briskie, H. G. Dowling and an anonymous reviewer for critical comments on the manuscript. This research was funded by a Brian Mason Trust grant and the Shirtcliffe Fellowship. A. Todd was supported by a Foundation for Research, Science and Technology Top Achiever Doctoral Scholarship.

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# HERPETOLOGICAL HUSBANDRY

*Herpetological Review*, 2004, 35(4), 361–364.


## A Comparison of Vitamin D-Synthesizing Ability of Different Light Sources to Irradiances Measured with a Solarmeter Model 6.2 UVB Meter

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Recognition of nutritional metabolic bone disease (= nutritional secondary hyperparathyroidism) in herpetocultural collections in recent decades has led to an interest in measuring ultraviolet B (UVB) radiation (280–315 nm) in natural light and in vivaria illuminated with artificial lamps. UVB facilitates photoisomerization of 7- dehydrocholesterol (pro $D_3$) to previtamin $D_3$ (pre$D_3$) which in turn is thermally isomerized to vitamin $D_3$ (vit$D_3$) (Chen 1999;

TABLE 1. Demonstration of reciprocity between the UVB dose and the percent of product formation in ampules of 7-dehydrocholesterol between 30 minutes and 120 minutes. The UVB source was a Westinghouse FS 20/T12 fluorescent lamp. Irradiance was measured with a Solartech 6.2 meter.

| IRRADIANCE ($\mu W/cm^2$) | EXPOSURE TIME (min) ($mJ/cm^2$) | DOSE | % PRODUCT SYNTHESIZED |
|---|---|---|---|
| 142 | 30 | 255 | 34.42 |
| 70 | 60 | 252 | 34.20 |
| 35 | 120 | 252 | 34.14 |

TABLE 2. Various characteristics of natural light in Boston, Massachusetts (USA) (42°N) at different seasons and different times of the day. Irradiances were measured with a Solarmeter 6.2 radiometer (Solartech, Inc., Harrison Township, Michigan 48045). The regression equation relating the % of photoproducts formed to the dose is presented for each season. Ampules used to assess vitamin D-synthesizing ability of the light were exposed for 1 hour.

| DATE; TIME (EST) | IRRADIANCE ($\mu W/cm^2$) | DOSE ($mJ/cm^2$) | % PRODUCT SYNTHESIZED |
|---|---|---|---|
| **21-Jan-2003** | | | |
| 1100 | 42 | 151 | 0.17 |
| 1200 | 56 | 202 | 0.19 |
| 1300 | 52 | 187 | 0.25 |
| 1400 | 37 | 133 | 0.15 |
| 1500 | 20 | 72 | 0.06 |
| %Prod = 0.001 (Dose) - 0.020 ($r^2 = 0.8322$) | | | |
| **3-Mar-2003** | | | |
| 1130 | 120 | 432 | 0.66 |
| 1230 | 132 | 475 | 0.98 |
| 1330 | 124 | 446 | 0.85 |
| 1430 | 97 | 349 | 0.48 |
| 1530 | 50 | 180 | 0.16 |
| %Prod = 0.003 (Dose) - 0.351 ($r^2 = 0.9249$) | | | |
| **26-Jul-2003** | | | |
| 1100 | 260 | 936 | 5.2 |
| 1200 | 262 | 943 | 6.8 |
| 1300 | 275 | 990 | 7.4 |
| 1400 | 240 | 864 | 6.8 |
| 1500 | 215 | 774 | 6.5 |
| 1600 | 130 | 468 | 2.0 |
| %Prod = 0.009 (Dose) - 1.730 ($r^2 = 0.7650$) | | | |
| **24-Sep-2003** | | | |
| 1100 | 140 | 504 | 2.0 |
| 1200 | 180 | 648 | 2.7 |
| 1300 | 215 | 774 | 3.5 |
| 1400 | 185 | 666 | 3.2 |
| 1500 | 160 | 576 | 2.0 |
| 1600 | 95 | 342 | 1.2 |
| %Prod = 0.006 (Dose) - 0.803 ($r^2 = 0.9274$) | | | |

TABLE 3. Characteristics of four UVB emitters. Irradiances were measured with a Solarmeter model 6.2 radiometer (Solartech, Inc., Harrison Township, Michigan 48045). The regression equation relating the % of photoproducts formed to the dose is presented for each source. Ampules used to assess vitamin D-synthesizing ability of the UVB sources were exposed for 2 hours. The temperatures adjacent to the distances listed for the Westron 160 W spot were those measured after 2 hours.

| LAMP DISTANCE (cm) | IRRADIANCE ($\mu W/cm^2$) | DOSE ($mJ/cm^2$) | % PRODUCT SYNTHESIZED |
|---|---|---|---|
| BLACKLIGHT 350 BL | | | |
| 11 | 25 | 180 | 3.37 |
| 23 | 13 | 94 | 1.34 |
| 34 | 7 | 50 | 0.91 |
| 46 | 4 | 29 | 0.45 |
| %Prod = 0.019 (Dose) - 0.162 ($r^2 = 0.9775$) | | | |
| REPTISUN 5.0 | | | |
| 11 | 36 | 259 | 1.39 |
| 23 | 16 | 115 | 0.54 |
| 34 | 9 | 65 | 0.27 |
| 46 | 5 | 43 | <LD* |
| %Prod = 0.006 (Dose) - 0.116 ($r^2 = 0.9997$) | | | |
| ESU REPTILE DESERT 7% UVB | | | |
| 11 | 53 | 382 | 1.79 |
| 23 | 25 | 180 | 0.68 |
| 34 | 14 | 101 | 0.35 |
| 46 | 8 | 58 | <LD* |
| %Prod = 0.005 (Dose) - 0.209 ($r^2 = 0.9969$) | | | |
| WESTRON 160 W SPOT | | | |
| 30 (45°C) | 173 | 1246 | 4.39 |
| 42 (38°C) | 92 | 662 | 2.68 |
| 88 (29°C) | 24 | 173 | 1.07 |
| 127 (26°C) | 12 | 86 | 0.82 |
| %Prod = 0.003 (Dose) + 0.562 ($r^2 = 0.9992$) | | | |

* less than lower limit of detection

Holick 2004). The irradiance of UVB is related to the rate of $preD_3$ production and hence indirectly to the rate of $vitD_3$ synthesis.

Spectroradiometers can accurately measure the irradiance of UVB from a light source. However, they are expensive and relatively difficult to work with. Hand-held broadband radiometers are less expensive and easy to use but may indicate irradiances that are significantly different from the actual values. Gehrmann et al. (2004) examined three types of broadband radiometers and showed that the meters indicated different levels of irradiance from the same light source. The determination of the vitamin $D_3$-photosynthesizing ability of a light source can serve as an independent measure of UVB irradiance. Thus, the percentage of photoproducts formed from $proD_3$ after exposure to the light for a specified time period allows for the comparison of irradiances from the different meters.

We recently became aware of an inexpensive broadband UVB meter manufactured by Solartech, Inc. (Harrison Township, Michigan 48045) as model no. 6.2, reading UVB between 280 and 320 nm and with a resolution of 1 $\mu W/cm^2$. In order to facilitate comparisons with the three meters described by Gehrmann et al. (2004) (Gigahertz-Optik, Inc. [Newburyport, Massachusetts 01950], UVP, Inc. [Upland, California 91786], and Spectronics Corp. [Westbury, New York 11590]) we correlated irradiances and associated doses from natural sunlight and various lamps with in vitro $vitD_3$-synthesizing ability.

Measurements of UVB in natural light at different times of the day were made in Boston, Massachusetts during the four seasons of 2003. UVB irradiance readings for the 1 hour during which $proD_3$-containing ampules were exposed were recorded at the start, middle, and end of the hour and then averaged. Readings were made at a solar angle of 80°. Irradiances from the three 20-watt fluorescent lamps were recorded at various distances below the midpoint of the lamp length; the meter was slowly moved about at this location to achieve the maximum reading. Irradiances were recorded at various distances below the center of the light circle produced by the Westron mercury vapor lamp. $ProD_3$ ampules were placed at these positions and exposed for 2 h to maximize conversion.

After exposure to the light source, the boron-silicate ampules that had contained 50 µg of $proD_3$ dissolved in one ml of ethanol

were analyzed by High Performance Liquid Chromatography (HPLC) for $proD_3$ and UVB-induced photoproducts ($preD_3$, tachysterol, and lumisterol) and $vitD_3$. A Waters 501 HPLC pump was used in conjunction with a Waters 490E multiwave detector set at 260 nm. The column was Econosphere silica, 5 µm, 250 x 4.6 mm (Alltech Associates, Inc, Deerfield, Illinois). The mobile phase was 8% ethyl acetate in hexane with a flow rate of 1.8 ml/min. Three replicates per ampule were analyzed and the percent of photoproducts and $vitD_3$ synthesized was calculated (see Gehrmann et al. 2004 and Webb et al. 1988 for details of the HPLC procedure).

We wished to verify that comparisons of photoproduct formation between the natural light ampules exposed for one hour and those exposed to the lamps for two hours were valid. The reciprocity law (Parrish et al. 1978) states that the UVB effect on photoproduct formation is not from irradiance *per se* (i.e., rate of energy delivery as watts), but rather the total energy (joules) delivered during a given time period which is the dose [dose ($mJ/cm^2$) = irradiance ($\mu W/cm^2$) x time (seconds) ÷1000]. We selected three different exposure times (30, 60, and 120 minutes) and adjusted the irradiance from a lamp, by changing the distance, such that the delivery dose was the same for all three periods. If the law was applicable to this study, the percent of photoproducts formed in the ampules should be the same for the three exposure times.

Results (Table 1) demonstrate the validity of the reciprocity law and justify comparisons of doses and associated photoproduct synthesis between ampules exposed to natural light for one hour with those exposed to various lamps for two hours. This idea is embodied in the regression equations relating the percent of photoproduct formed to the UVB dose (not the irradiance) that are given in Tables 2 and 3. Table 2 shows the irradiances of UVB in natural light in Boston measured with a Solartech 6.2 meter, and the associated doses, and relates them to the percent of photoproducts formed in ampules after one hour of exposure. Daily and seasonal trends are evident. Table 3 contrasts the UVB irradiances at different distances from four lamp types commonly used in herpetoculture and indicates their ability to produce $vitD_3$ photoproducts in ampules exposed for two hours. It is evident that the same irradiance and associated dose from different lamps can produce different quantities of photoproducts. Not all wavelengths within the UVB band are equally effective in producing $preD_3$ from $proD_3$. The greater the percent photoproduct formed at a given dose, the greater the concentration of UVB energy clustered around the most effective wavelength of 295 nm. This is clearly demonstrated by the Westinghouse FS 20 UVB lamp in Table 1. The percent of photoproducts formed is considerably higher than for the other light sources described in this article because of the high concentration of UVB close to 295 nm.

Evaluating the significance of $vitD_3$-synthesizing potential for herpetocultural purposes remains largely unexplored. Systematic studies showing the effect of latitude on the ability of natural light to form photoproducts in ampules could serve as an estimate of UVB requirements for various species in captivity taken in the context of their natural history and habitat preferences. Scattered reports indicate significant latitudinal effects on $vitD_3$-synthesizing ability. Webb et al. (1988) reported ampule conversions of 3% in Los Angeles, California (34°N) and 10% for Puerto Rico (18°N), both in January. Gehrmann et al. (2004) report conversion at about 11% at noon in Boyd, Texas (32°N) in September. Conversion at Iquitos, Peru (3°S) was about 15% in February (Gehrmann, unpubl. data). Ampule photoproduct formation was characterized throughout the year in Edmonton, Canada (52°N) by Webb et al. (1988). Additional examples can be found in Chen (1999) and Holick (2004).

Knowing the UVB requirements for a species studied in captivity allows for more specific recommendations. For example, Ferguson et al. (2002) reported that lamps that produce conversions from 0.52% to 1.32% after a 2 h exposure when used for 12 h per day, facilitate the production of viable hatchlings in the panther chameleon. Conversion percentages above or below these values resulted in reduced hatchability of viable eggs. Referring to Table 3 and using the regression equations, we see that a blacklight with an irradiance of between 5 and 11 $\mu W/cm^2$, a Reptisun 5.0 between 15 and 33 $\mu W/cm^2$, an ESU Desert 7% between 20 and 42 $\mu W/cm^2$ and a Westron spot between 8 and 35 $\mu W/cm^2$ will produce conversions within the 0.52% to 1.32% range. It is suggested that further studies relating UVB irradiance to husbandry and reproduction in reptile species, especially lizards, will contribute to their captive welfare.

The percent of photoproduct formation in ampules can serve as a reference to doses and associated irradiances measured with other meters. For example, from Table 3 we see that for a Sylvania 350 blacklight the amount of photoproduct formed is 1.34% when the irradiance is 13 $\mu W/cm^2$. Using the data in Gehrmann et al. (2004) it can be calculated that 1.34% is associated with an irradiance of 7 $\mu W/cm^2$ measured with a Gigahertz-Optik meter, 80 $\mu W/cm^2$ measured with a UVX meter, and 17 $\mu W/cm^2$ measured with a Spectroline DM 300N meter.

The Solarmeter sensor and processor are combined as a single unit. Because the sensor is located about 10.5 cm above the meter bottom, it cannot be used to directly measure the irradiance at the substrate level in an enclosure. Nevertheless, this meter will be useful for many purposes, including monitoring the UVB output of various lamps or checking the attenuation of UVB by various materials.

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