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Photobiosynthetic Opportunity and Ability for UV-B Generated Vitamin D Synthesis in Free-Living House Geckos (*Hemidactylus turcicus*) and Texas Spiny Lizards (*Sceloporus olivaceous*)

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The opportunity and ability to photobiosynthesize vitamin D_3 by exposing skin to ultraviolet-B (UVB) irradiation from the sun was compared using the nocturnal/crepuscular Mediterranean House Gecko *Hemidactylus turcicus* and the diurnal Texas Spiny Lizard *Sceloporus olivaceous*. Texas spiny lizards had a greater opportunity for photobiosynthetic production of vitamin D_3 than geckos. This was revealed by vitamin D_3 photoproduct production in models (ampoules containing an alcohol solution of vitamin D_3 precursor) placed at locations inhabited by free-living lizards at similar times of occupancy. Alternatively, geckos seemed able to maximize their limited photobiosynthetic opportunity with a higher rate of conversion of provitamin D_3 to photoproducts. This was revealed by photoproduct conversion in patches of lizard skin exposed to ultraviolet lamps in the laboratory. Stomach-content analysis showed the spiny lizards to have dietary sources of vitamin D_3, the geckos may or may not. This is the first documentation that mostly nocturnal geckos may rely on photobiosynthesis of vitamin D_3 and that they might have a more sensitive mechanism than diurnal lizards to compensate for their limited exposure to natural UVB radiation. Future studies should investigate sexual, seasonal, age, and species differences in photobiosynthetic opportunity and ability.

SINCE Cowles and Bogert's (1944) groundbreaking study on reptile thermoregulation, numerous studies have implied that basking behavior functions solely as a method of thermoregulation (Huey and Slatkin, 1976; Huey and Webster, 1976; Sievert and Hutchison, 1988). However, sun exposure (specifically ultraviolet-B radiation or UVB; 290–320 nm) has been shown in several vertebrates to be necessary in the production of vitamin D_3 via the cutaneous photobiosynthesis of previtamin D_3 from provitamin D_3 (Webb and Holick, 1988; Webb et al., 1989; Allen et al., 1994). Once previtamin D_3 is produced under the stimulus of UVB irradiation, it is thermally isomerized to vitamin D_3. Only the second step in the process is temperature dependent. Panther chameleons seek out high UVB doses more readily when deprived of dietary vitamin D_3 (Jones et al., 1996). In addition, turtles have been shown to bask for unknown reasons other than thermoregulation (Pritchard and Greenhood, 1968; Manning and Grigg, 1997). Allen et al. (1994) suggested that rapidly growing juvenile Komodo dragons may require UVB irradiation as a supplement to their dietary vitamin D_3 source. Even diurnal basking day geckos seem to require UVB exposure in addition to vitamin D_3 in their diet (Allen et al., 1996). Thus, there seem to be functions for basking other than thermoregulation.

Vitamin D_3 is a nutrient that serves many physiological functions, the most recognized of which are the maintenance of blood calcium levels (Webb and Holick, 1988), calcium metabolism (How et al., 1994), and maintenance of healthy bones (Lu et al., 1992). The vitamin can be obtained not only from photobiosynthetic production but also directly from dietary sources (Holick et al., 1995). Since many prey items are thought to be low in vitamin D_3 content (How et al., 1994), photobiosynthesis may be more important than originally thought.

Recent data demonstrate variability in the ability of different terrestrial organisms to photobiosynthesize vitamin D_3. Rats possess the ability to synthesize vitamin D_3, whereas dogs and cats cannot (How et al., 1994). Unfortunately, a thorough comparative study of wild species has not been performed.

We report the first combined field and laboratory investigation of photobiosynthetic opportunity and ability for UVB-generated vitamin D synthesis in reptiles. Two lizards with very different ecologies were investigated: a nocturnal/crepuscular House Gecko (*Hemidactylus turcicus*) and the diurnal Texas Spiny Lizard (*Sceloporus olivaceous*).

The house gecko is a small (10–12 cm) lizard that was introduced into the United States from the Mediterranean area. In the United States, it

breeds from March to July and lays eggs from April to August. It ranges from southern Louisiana to southern Texas, is found in patches in north Texas, and is also found in peninsular Florida. It is most often found on lighted walls eating insects that are attracted by lights (Anonymous, 1997).

The Texas spiny lizard (TSL) is a large (19–29 cm) diurnal lizard that breeds and lays eggs during the spring and summer. It ranges from south-central Oklahoma to Mexico and can be found in trees such as the mesquite, live oak, and others (Blair, 1960).

The photobiosynthetic opportunity of the two species was assessed by using in vitro models as described in methods. Photobiosynthetic ability was assessed by exposing skin samples to UVB irradiation for timed intervals. Finally, vitamin D_3 levels of the stomach contents of free-living animals were analyzed for both species.

Materials and Methods

Ultraviolet light exposure could not be estimated either directly or indirectly when simultaneously observing a lizard in the field without disturbing it and altering the rest of its activity or habitat use pattern. Thus, assessment of the opportunity for photobiosynthesis was achieved indirectly by recording the exact locations of a free-living specimen in the field, monitoring it throughout a diel cycle (focal day), and placing ampoules made of UV conducting glass containing 50 μg per ml of provitamin D_3 (vitamin D_3 precursor) dissolved in 100% ethanol (hereafter referred to as in vitro models or models) in the exact location over the same time period on a subsequent day (model day). The amount of photoproduct (previtamin D_3 plus vitamin D_3) in the exposed models was used as a relative index of photobiosynthetic opportunity; the more conversion of provitamin D_3, the more opportunity.

In July 1997, the lizards were located and observed for one cycle at each field site (all field sites were urban sites located in Tarrant County, Texas) to increase familiarity with their behavior. Then, three specimens of each species were chosen for further observation. Each lizard was followed for one diel cycle, and its location was documented every 15 minutes or after each move. When necessary, a photograph of the lizard and location was taken to ensure accuracy of documentation. All documentation was used as a guide to place a model at the exact location of the specimen on the next available climatically similar day (usually one or two days later). A model was moved along sequential locations for three hours and then replaced by a fresh model for another three hours (rate of photoconversion in a model slows after three hours; unpubl. data). Thus, three or four models were used to retrace a single activity cycle of one lizard. Irradiance similarity between focal and model days was monitored by exposing and replacing one model every three hours in a common neutral site on both days.

Because previtamin D_3, once formed, spontaneously converts to several photoproducts (mostly vitamin D3), the analysis of total photoproduct formation is simplified if the conversion is retarded. Because the rate of conversion to vitamin D3 is temperature dependent, models were kept below 22 C during exposure period by fastening them to frozen Blue Ice bags. Bags were changed as necessary throughout the cycle to maintain the low temperature. Cooling was not critical for accuracy of the data because total photoproduct (previtamin D_3 plus vitamin D_3) is equivalent to previtamin D_3 initially formed. After three hours of exposure, models were replaced, wrapped in foil, stored on ice for the remainder of the field day, and then placed in a −70 C freezer for later analysis.

An apparatus was constructed to place models at documented locations that were difficult to reach due to height. Polyvinyl conduit was cut into 1.54-m lengths that could be attached to each other. A styrofoam rectangle with a concave side was affixed to one end of the conduit and a frozen Blue Ice bag placed in the concavity. A model was then placed on the Blue Ice bag and held into position with a rubber band.

Vitamin D_3 photobiosynthesis occurs in the skin of vertebrates (Holick et al., 1995). UV (at least UVB) irradiation does not penetrate the skin to deeper tissue layers (Porter, 1967). Therefore, assessment of the ability for photobiosynthesis was achieved by analyzing UVB-exposed skin samples. Four specimens of each species were captured from the field and stored for up to 12 h in a cool (8 C), dark location. Each was weighed, measured, then pithed, and the brain macerated to ensure instantaneous death. Skin patches were removed immediately from the dorsal surface of the specimen and sectioned into four 5 × 5 mm rectangular samples using a razor blade and a 5 × 5 mm paper template. The samples were kept moist throughout the process using physiological saline. They were then placed in a petri dish 34.29 cm under a UV lamp (Spectroline Medium Wave UV 320 nm) and exposed to UVB radiation for four time intervals. A control received no exposure (time zero), whereas the other samples received 20, 40, or 60 min of UV exposure. The dish was

TABLE 1. PROVITAMIN D_3 REMAINING IN CONTROL VIALS ON FOCAL VERSUS VIAL DAY. Values are percent ± SD (n). Because there were six comparisons, α was adjusted using Bonferroni correction from $P < 0.05$ to $P < 0.007$. Gecko 3 failed the normality test; therefore values are given.

Lizard	Focal Day	Vial Day	t	P
Gecko 1	100.2 ± 0.920 (4)	99.4 ± 1.212 (4)	1.02	0.3477
Gecko 2	99.8 ± 0.252 (4)	100.5 ± 1.537 (4)	−0.692	0.4849
Gecko 3	99.1 (4)	98.7 (4)	22.0	0.3429
TSL 1	98.0 ± 2.156 (4)	98.4 ± 0.707 (4)	−0.353	0.7364
TSL 2	95.2 ± 6.10 (5)	96.8 ± 3.24 (4)	−0.449	0.6668
TSL 3	93.3 ± 5.41 (8)	94.4 ± 4.70 (4)	−0.329	0.7487

rotated every 5 min to compensate for positional effects on the samples. Once irradiated, samples were placed in labeled vials and stored in a −70 C freezer for later analysis. UVA and B irradiation measurements were taken prior to exposure using Spectroline UV meters (Models DM-300N medium wavelength 300 nm and DM-365N long wavelength 365 nm, respectively); irradiances were 21 uW/cm² (UVA) and 90 uW/cm² (UVB). In vitro models were exposed one hour to these conditions for comparison to the skins. All models, skin samples, and stomach contents were analyzed for provitamin D_3, previtamin D_3, and vitamin D_3 as previously described (Holick et al., 1981).

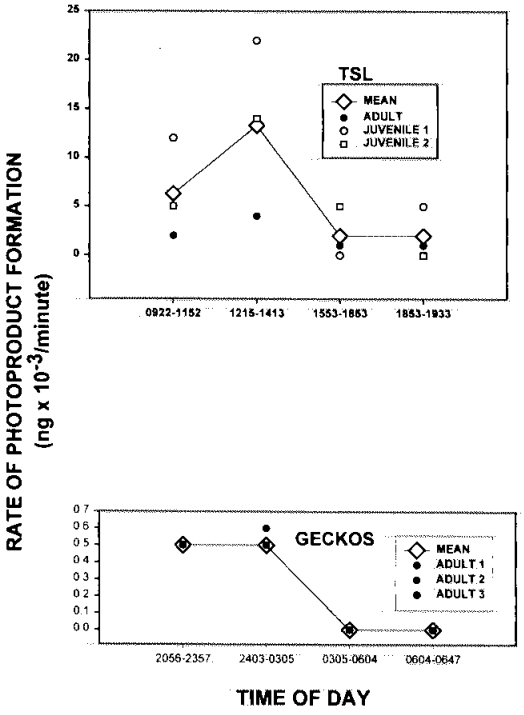

Fig. 1. Rate of photoproduct (previtamin D_3 plus vitamin D_3) formation at different times of day for in vitro models at the free-living locations of three Texas spiny lizards (TSL) (*Sceloporus olivaceous*) and three house geckos (*Hemidactylus turcicus*) in Fort Worth, Texas in July and August 1997. TSL locations photoproduct formation was greater than that of the gecko locations. Maximum TSL location photoproduct formation occurred at midday.

Statistical analyses were performed using Microsoft Excel version 7.0 and Systat version 6.0. Transformations were made where necessary to normalize the data. Where skin and stomach sample values were below the limit of detection, the maximum possible value was estimated by dividing the minimum detection level by sample mass. Detection limits were 0.5 ng for vitamin D_3, 1.0 ng for previtamin D_3, and 1.0 ng for provitamin D_3.

RESULTS

Opportunity for photobiosynthesis.—Comparison (*t*-test) of the photoconversion of the control models for the focal and model days revealed no significant differences between days (Table 1). The rate of photoproduct (previtamin D_3 plus vitamin D_3) formation was higher at the Texas spiny lizard (TSL) locations than at gecko locations (minimum TSL= 0.0009 ng/min and maximum gecko = 0.0004 ng/min; Fig. 1). Maximum levels of photoproduct formation occurred at midday for all three TSL locations and minimum levels tended to occur at the later hours.

Ability for photobiosynthesis.—All TSL and gecko skin samples started with less than 10% photoproduct conversion [100(previtamin D_3 + vitamin D_3)/(provitamin D_3 + previtamin D_3 + vitamin D_3)] except one gecko, which started with 58% conversion. To standardize time 0 to a low value, this gecko was eliminated from statistical analysis. There was a significant trend for an increase in mean percent photoproduct conversion with time for both species combined (time effect $P < 0.05$, $F = 9.81$, df = 3; repeated measures ANOVA; Fig. 2). However, the conver-

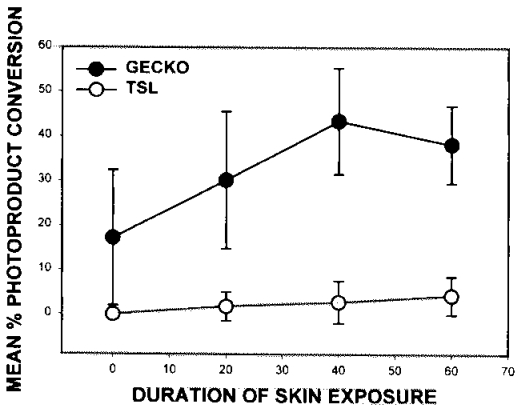

Fig. 2. Mean percent of photoproduct conversion versus duration of skin exposure for 5 × 5 mm patches of Texas spiny lizard (TSL) and gecko skin exposed to a UV bulb (90 uW/cm^2 UVB). Vertical bars show two standard errors. There was a significant trend for increase in photoproduct conversion (time effect) as well as a significant difference between the two species (group effect; time-group interaction). See text for statistical detail. In this experiment, the conversion in TSL skin was weak and not significant.

sion of previtamin D_3 in TSL was weak and not significant by itself. There was a significant difference between the photoproduct formation of the two species over time (group effect $P < 0.05$, $F = 36.18$, df = 1; time-group interaction $P < 0.05$, $F = 6.84$, df = 3; repeated measures ANOVA). Mean percent conversion for the in vitro models was 20.2% (SD ± 2.2; n = 2). Model values were not significantly different from those of geckos ($\bar{x} = 31.3$, SD ± 8.1; n = 3) but were significantly higher than those of TSL ($\bar{x} = 5.7$, SD ± 3.5, n = 4; $P < 0.05$ ANOVA and Tukey HSD) at one hour of exposure. The control skin patches of the TSL contained slightly but not significantly more provitamin D_3 than those of the geckos (TSL $\bar{x} = 34.7$, SD ± 19.7 ng, n = 4; gecko $\bar{x} = 20.8$, SD ± 13.7 ng, n = 4; $P = 0.29$ t-test).

Stomach contents.—There was no significant difference in vitamin D_3 levels between gecko and TSL stomach contents ($P = 0.3734$, $t = 0.962$, df = 6). Mean maximum possible vitamin D level level in gecko stomach content was less than 4.43 ng/g. (SD = ± 2.76, n = 4), whereas mean in TSL stomach contents was 6.98 ng/g. (SD ± 4.53, n = 4). TSL stomachs contained mostly green larval lepidopterans, whereas gecko stomachs contained mostly adult lepidopterans and coleopterans.

Discussion

As expected the diurnal Texas spiny lizard had a greater opportunity for photobiosynthesis of vitamin D_3 than the house gecko (Fig. 1). The only apparent opportunity for photobiosynthesis in the nocturnal/crepuscular house gecko was during dusk and/or dawn (crepuscular) exposure. The presence of photoproduct formation in models retracing gecko activity at midnight is surprising and may be attributed to artifact since the conversion is very low and no known source of UV was present at that hour. Since all nights were clear and the sun did not set completely until 2130, the sun cannot be ruled out as a possible factor responsible for the presence of some photoproduct formation during the first gecko time interval.

Geckos located on west-facing walls seemed to emerge earlier (at dusk) than those on east-facing walls, and those on the east-facing walls seemed to remain active longer (into dawn) than those on west-facing walls, but these observations were not quantified. This is what would be expected if geckos were adjusting their activity cycle to obtain morning or evening sun exposure. Although most geckos have been generally assumed to be nocturnal and exposed to very little sunlight or opportunity for vitamin D_3 photobiosynthesis, Frankenberg (1978) observed significant diurnal activity in several gecko species (including *H. turcicus*) during certain times of the year.

The gecko's capacity to produce vitamin D_3 was significantly higher than that of the TSL (Fig. 2). Because concentrations of provitamin D_3 were similar, this was not a factor in their higher capacity, which may result from increased skin absorptivity of UVB. Their sensitivity may be an adaptation that allows the gecko to maximize its limited exposure to UVB, or photobiosynthetic opportunity. Because the TSL has ample opportunity for basking, there is no need to rapidly convert a large percentage of its provitamin D_3 to photoproducts. It is interesting that one gecko had already converted 58% of its provitamin D_3 prior to experimental irradiation.

Because TSL and geckos are active at different times with probable differences in their body temperatures during these times and knowing that the photobiosynthesis of vitamin D includes a temperature-sensitive step (conversion of previtamin D_3 to vitamin D_3, Tian et al., 1993), it would be interesting to see whether these species have different optimal temperatures for formation of vitamin D_3. Holick et al. (1995) found a lower rate of vitamin D_3 for-

mation at lower temperatures (5 C) than higher temperatures (25 C) in the skin of *Iguana iguana.*

The TSL had measurable vitamin D_3 in its stomach contents. Reanalysis with larger samples of gecko stomach contents is necessary to see whether measurable dietary levels of vitamin D_3 occur and whether they differ between the species.

We must emphasize that in vitro models similar to those used in this study provide an excellent way to measure relative availability of photobiosynthetically active light in the environment. It provides no good information on the absolute levels of photobiosynthetic production for an organism that may occupy the location of a model at a similar time when the model is exposed to the sun. The rate and absolute percent of conversion of provitamin D_3 to photoproducts is not the same for alcohol solutions and lizard skin (Holick et al., 1995; this study). Furthermore, probably due to factors such as UVB absorptivity (Porter, 1967) and provitamin D_3 concentration, potential synthesis in a patch of skin varies. So, studies combining models and animal skin samples will provide the best clues to the relative importance of endogenous production of vitamin D_3 in animals of different life stage and species. This should help us understand the role of basking for functions other than thermoregulation.

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