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# Digestive Efficiency in a Temperate Herbivorous Reptile, *Gopherus polyphemus*

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**Digestibility and passage time were measured in *Gopherus polyphemus* fed leaves of the legume *Aeschynomene americana*. Intake was held constant relative to body mass. Mean apparent digestive efficiencies were 68% of organic matter, 61% of energy, 73% of cell walls, and 71% of nitrogen. Mean passage time was 13 d. Body mass, within the range of 3.3–7.9 kg, had no effect on digestibilities or passage time. Potassium permanganate lignin was not an acceptable indigestible dietary marker; in vitro indigestible cell walls was an accurate indigestible marker. *Gopherus polyphemus* is as capable of maintaining an efficient gut cellulolytic microflora and degrading the fiber fraction of its diet as are tropical reptiles.**

INTEREST in the nutrition of herbivorous reptiles has increased in recent years. Several studies on digestive efficiency have demonstrated that tropical herbivorous reptiles rely on a cellulolytic microflora in their digestive tracts to utilize the cell wall (or cellulose, hemicellulose and lignin) fraction of their diets. Significant degradation of dietary cellulose occurs in the digestive tracts of *Chelonia mydas* (Bjorndal, 1979), *Sauromalus hispidus* and *S. varius* (Voorhees, 1981), *Geochelone gigantea* (Hamilton and Coe, 1982), *Iguana iguana* (Troyer, 1982), *Conolophus subcristatus* (Christian et al., 1984), and *Geochelone carbonaria* and *G. denticulata* (Bjorndal, in prep.). All tropical herbivorous reptiles examined to date harbor a cellulolytic microflora.

It is not clear whether temperate reptiles can and/or do rely on cellulolytic microflora to the extent found in tropical reptiles. The only study of cellulose digestion in a temperate reptile (*Sauromalus obesus*) gave contradictory results, but suggested that, if a cellulolytic microflora is present, its activity is very low and unlikely to yield significant benefits to the host lizard (Nagy, 1977).

The greater environmental temperature fluctuations and the extended periods of hibernation and/or aestivation that temperate herbivorous reptiles undergo make it questionable whether a temperate reptile could maintain an efficient gut microflora that would contribute significantly to the reptile's energy balance. In order to answer this question, I conducted a feeding trial with the gopher tortoise, *Gopherus polyphemus*, on a high fiber, foliage diet. Gopher tortoises range throughout the southeastern United States and feed almost entirely on vegetation (Carr, 1952). The tortoises for this study were collected in north Florida, where gopher tortoises remain in their burrows for 4–5 mo during winter (Carr, pers. comm.). In addition to determining whether cellulose is fermented to a significant degree in *G. polyphemus*, I measured the digestibilities of other nutrients in the gopher tortoise and tested the accuracy of two indigestible dietary markers.

## METHODS

*Feeding trial.*—The seven gopher tortoises, *G. polyphemus*, in the feeding trial ranged in body mass from 3.3–7.9 kg; both sexes were represented. The diet consisted of leaves removed from dried *Aeschynomene americana*, a legume native to Florida. Leaves were picked off stems individually in order to ensure a homogeneous diet. Aeschynomene is very palatable to tortoises.

Tortoises were separated into individual, outdoor, wooden pens that measured 1.2 × 2.0 m. The tortoises were out of sight of each other throughout the trial. Every day for 5 wk, each tortoise was given an amount of food equal to 0.22% of its body mass (dry matter/live mass). This amount is less than the predicted ad libitum feeding level (Bjorndal, 1985a) and was chosen to keep the animals feeding regularly. In order to rehydrate the hay diet, leaves were soaked in dechlorinated water for 45 min before being presented to the tortoises. Each animal consumed its entire ration each day within 90 min. All tortoises maintained their mass throughout the trial. Ambient temperature in

the pens varied from 20.5–30.5 C. Tortoises were able to bask; shade was always available. Drinking water was not provided during the trial.

The number of days until first appearance of aeschynomene in the feces was used as an estimate of passage time, or more specifically of transit time (the time from ingestion to first appearance in the feces). The tortoises had been fed a mixture of grasses—St. Augustine (*Stenotaphrum secundatum*), centipede (*Eremochloa ophiuroides*) and bahia (*Paspalum notatum*)—before the trial. Feces from the grass diet did not appear to be mixed with the feces from the aeschynomene diet, and the two types of feces were readily distinguishable. Diapers were fastened to the animals 2 d after aeschynomene feces first appeared (12–18 d from the start of the trial, depending on passage time in the tortoise). Feces were collected for 2 wk. Tortoises were maintained on the experimental diet throughout the collection period. The diapers consisted of plastic bags covered with cloth and were hooked onto elastic straps (threaded through holes drilled in the anterior carapace and plastron) that held the diaper firmly in place.

Digestive efficiencies were calculated by the equation:

$$([\text{intake of X} - \text{output of fecal X}]/[\text{intake of X}]) \times 100$$

where intake and output are measured in grams (except energy is kJ) and X is a diet component (e.g., organic matter, energy, cell walls). Intake and output were calculated as daily intake times 14 d and as the sum of feces produced over 14 d, respectively. This equation yields apparent digestive efficiencies, or the net loss of a nutrient as it passes through the digestive tract. Dry matter digestibility was not calculated because of the possibility of sand contamination, which would result in underestimates of digestibility. The accuracy of potassium permanganate lignin and in vitro indigestible cell walls (IVICW) as indigestible markers was determined by using the above equation to measure their digestibility.

*Sample preparation and chemical analyses.*—Feces were removed once a day for 2 wk after the diapers were attached. Daily fecal production was fairly constant with the consistent feeding regime. Uric acid pellets were removed from the feces. The feces were weighed, dried to constant mass at 60 C (about 24 h) and weighed again. Diet samples were collected daily as the diets for the tortoises were prepared. Diet samples were weighed before and after being soaked in dechlorinated water for 45 min, then dried to constant mass at 60 C and weighed again.

Dried diet samples were combined and fecal samples were combined for each tortoise for chemical analyses. The samples were ground through a 1 mm screen in a Wiley mill. A portion of each sample was dried at 105 C to determine percent dry matter (DM) and then ashed in a muffle furnace for 3 h at 500 C to determine percent organic matter (OM).

Composition of food and feces was determined for a series of definitive fractions. That is, the fractions are defined by the procedure used to obtain them, and not by the precise chemical composition of the fractions themselves. Cell walls (or neutral detergent fiber ash-free, NDFA) were measured by the Van Soest technique (Goering and Van Soest, 1970) as modified by Golding et al. (1985). Analyses of acid detergent fiber (ADF), potassium permanganate lignin, and in vitro indigestible cell walls (IVICW) followed Goering and Van Soest (1970). IVICW is a measure of the percentage of cell walls not degraded during a prolonged (96 h) in vitro fermentation with rumen microflora and is composed primarily of highly-lignified cellulose and cutin. Successful use of IVICW as a marker depends on this indigestible fiber residue not being degraded by microflora in other herbivores.

Energy content of food and feces was determined in a bomb calorimeter following standard procedure (Parr Instr. Co., Tech. Man. #130, 1960). Total (Kjeldahl) nitrogen was measured with a block digester (Gallaher et al., 1975) and an automated Technicon analyzer (Hambleton, 1977).

In addition, at the end of the feeding trial, feces were collected from tortoises included in the trial and from two tortoises not in the trial that had been feeding on a mixture of grasses and forbs. The grass/forb diet was a lower quality diet, with lower digestibility by rumen microbes, lower nitrogen content and higher cell wall content than aeschynomene leaves. The feces were analyzed for pH to 0.5 units with pH strips (ColorpHast, E. Merck Co.), and volatile fatty acids (VFA). For VFA analysis, samples of known mass and volume were preserved in 5% (w/w) phosphoric acid. Samples were kept frozen until analyzed. When thawed, the samples were shaken and centrifuged, and 1 $\mu$l of the

TABLE 1. DIET COMPOSITION AND DIGESTIVE EFFICIENCIES OF *Gopherus polyphemus* FEEDING ON LEAVES OF *Aeschynomene americana*. For diet composition: % dry matter (DM) of diet was measured after being soaked in water; organic matter (OM) is as % DM; energy is kJ/g OM; cell walls, acid detergent fiber, nitrogen, potassium permanganate lignin and in vitro indigestible cell walls (IVICW) are all as % OM. Digestibility equals percent net loss of nutrient from food to feces, mean ± standard deviation (individual values for tortoises #1–7, successively).

| | Diet composition | Digestibility (%) |
|---|---|---|
| Dry matter | 13.9 | — |
| Organic matter | 94.1 | 68 ± 2.6 |
| | | (69 69 69 68 71 70 63) |
| Energy | 21.3 | 61 ± 3.2 |
| | | (60 63 62 62 64 64 55) |
| Cell walls | 43.0 | 73 ± 1.2 |
| | | (75 74 73 73 73 73 71) |
| Acid detergent fiber | 24.6 | 62 ± 0.8 |
| | | (62 63 63 63 62 63 61) |
| Nitrogen | 5.3 | 71 ± 2.1 |
| | | (71 70 73 71 73 72 67) |
| Lignin | 7.5 | 34 ± 3.7 |
| | | (29 32 35 33 33 41 33) |
| IVICW | 9.8 | 0.3 ± 0.2 |
| | | (.3 .3 .3 .5 .4 .3 −.1) |

supernatant fluid was injected into a Hewlett Packard Model 5880A chromatography system equipped with auto-sampler and electronic integrator.

## RESULTS AND DISCUSSION

*Digestive efficiencies and passage time.*—The chemical composition of the diet (leaves of aeschynomene) and digestive efficiencies of seven gopher tortoises on this diet are given in Table 1. Christian et al. (1984) reported high individual variability in digestibilities of energy (coefficient of variation [CV] = 0.26) and cellulose (CV = 0.43) for five *Conolophus subcristatus*. Individual variation was much lower in gopher tortoises for energy digestibility (CV = 0.05) and digestibility of acid detergent fiber, the fraction most equivalent to cellulose (CV = 0.01). Much of the variation came from one tortoise (#7, Table 1). The reduced individual variability measured in gopher tortoises may result from the facts that the tortoises were housed under more constant conditions than was possible for the iguanas, feces were collected for a longer time (14 vs 6 d) so that daily variation would be equalized, great care was taken in preparation of a homogeneous diet for the tortoises, and the tortoises were fed a fixed percentage of their body mass whereas the iguanas were fed ad libitum. This last factor could introduce great variability, particularly in a trial of such short duration. The CVs for tortoises calculated here may be lower than those of naturally feeding tortoises, because they were fed a fixed amount, and thus should not be extrapolated to free-feeding animals.

Mean passage time was 13 d (SD = 2.8, range 10–16 d). Mean dry matter percent of feces was 19.1 (SD = 2.3, range 16.7–23.2%).

The high digestibility of cell walls (Table 1) indicates that *G. polyphemus* does rely on a cellulolytic microflora to degrade cellulose and hemicellulose in its diet. Digestive capabilities of herbivorous reptiles are difficult to compare because techniques among the studies have varied, and different parameters were measured. Also, diets fed to the animals have had a wide range in chemical composition. In Table 2, data from digestive studies for turtles and tortoises are presented. The digestive efficiencies of *G. polyphemus* are among the highest recorded, particularly for the apparent digestibility of nitrogen. These high efficiencies are probably a result of the high nutrient quality of aeschynomene, especially in nitrogen content. However, they indicate that a temperate reptile can at least equal the digestive efficiency of tropical reptiles.

Intake, passage time, and digestibility are inter-related parameters. For any given diet, higher intakes generally result in shorter passage times and lower digestive efficiencies. Therefore, to test the effect of body size on digestive efficiency and passage time, intake was held constant relative to body size, to avoid confounding the variation due to size differences with those due to intake differences. The appropriate exponent to scale intake to body mass within a tortoise species is not known. It may be closer to 0.9 (K. Nagy, pers. comm.) than 1.0, which I used in this study. However, mass-specific intake (g food per kg body mass per day) of two tortoise species (*Geochelone carbonaria* and *G. denticulata*) did not change significantly with body mass (range 0.4–6.6 kg) when fed ad libitum on three different diets (Spearman rank correlation, alpha = 0.05; Bjorndal, in prep.).

Within the size range tested (3.3–7.9 kg), body

TABLE 2. MEAN DIGESTIVE EFFICIENCIES AND DIET COMPOSITION FOR TURTLES AND TORTOISES. Energy (E) is kJ/g organic matter (OM); cell walls (CW) and nitrogen (N) are as percent OM, except where noted.

| | Digestive efficiencies | | | | Diet composition | | | |
|---|---|---|---|---|---|---|---|---|
| | OM | E | CW | N | E | CW | N | References |
| *Gopherus polyphemus* | 68 | 61 | 73 | 71 | 21 | 43 | 5 | This paper |
| *Gopherus agassizi* | — | 54 | — | 21 | 17[a] | — | 1[b] | Nagy and Medica, 1986 |
| *Geochelone carbonaria* | 38 | 34 | 37 | 26 | 22 | 53 | 3 | Bjorndal, in prep. |
| *Geochelone denticulata* | 41 | 37 | 41 | 31 | 22 | 53 | 3 | Bjorndal, in prep. |
| *Geochelone gigantea* | 38 | 35 | 42[c] | — | 18 | 64[c] | — | Hamilton and Coe, 1982 |
| *Chelonia mydas*[d] | 65 | 58 | 78 | 54 | 19 | 59 | 4 | Bjorndal, 1980 |

[a] kJ/g dry matter.
[b] As % dry matter.
[c] Values are for holocellulose, which approximates cell walls minus lignin.
[d] 66 kg size class.

mass had no effect on any of the digestive efficiencies measured: organic matter, energy, cell walls, acid detergent fiber, nitrogen, potassium permanganate lignin or IVICW ($P > 0.05$, Spearman rank correlation). Likewise, passage time was not affected by body mass ($P > 0.05$, Spearman rank correlation).

Parmenter (1981) found no effect of body size on passage time in *Pseudemys scripta.* Hamilton and Coe (1982) found that body size did not significantly affect dry matter digestibility, but did have a significant effect on passage times in *G. gigantea.* In *Chelonia mydas,* digestibility of organic matter, energy, and nitrogen were affected significantly by body mass, but digestibility of cell walls was not (Bjorndal, 1980). However, the apparent discrepancy in these findings could be due to the fact that intake was not held constant relative to body size, and that the range of sizes tested varied among studies. In *Iguana iguana,* Troyer (1984a) found no effect of size on digestibility of dry matter, protein or cell walls, but she did find a significant effect of size on passage time. Although she fed each size class a constant amount, it is not clear whether the ratio of intake to body mass was constant among the three size classes.

*Indigestible markers.*—Indigestible markers are useful for measuring intake and digestibility in animals when total measurement of intake and/or feces is either impossible or inconvenient. Indigestible markers are also essential for tracing changes in nutrient composition of digesta along the alimentary canal.

I tested sulfuric acid lignin as an indigestible marker in green turtles and found that is was accurate; 99.2% of the dietary sulfuric acid lignin was recovered in the feces (Bjorndal, 1980). Because this laboratory method requires the use of asbestos fibers, I can no longer use the technique. In this study I tested two diet components for their accuracy as indigestible markers—potassium permanganate lignin and in vitro indigestible cell walls (IVICW). Mean digestibility of potassium permanganate lignin was 34% (Table 1). That is, only 66% of dietary potassium permanganate lignin was recovered in the feces. If potassium permanganate lignin had been used as an indigestible marker in this study, digestive efficiencies would have been significantly underestimated. IVICW, however, proved to be an excellent marker, with a digestibility of only 0.3%, or a recovery of 99.7%.

Lignin is almost certainly not digested (although it may be partially soluble) as it passes through the digestive tract, because the only known pathways of lignin degradation require oxygen (Van Soest, 1982). However, it is important to remember that the techniques used to measure lignin are only crude approximations. Reports of digestion or "production" of lignin along the digestive tracts of mammals (Van Soest, 1982) and the report here of digestion of potassium permanganate lignin, probably refer to the degradation or production of contaminants in the crude lignin fraction—not to lignin itself. The fact that values for sulfuric acid lignin and potassium permanganate lignin are rarely the same for any given sample, and not expected to be, underscores the fact that these lignin fractions are definitive fractions.

These results indicate that, at least for *G. polyphemus* feeding on aeschynomene, potassium permanganate lignin is not an acceptable indigestible marker and that IVICW is an accurate marker. I obtained similar results when testing these two markers with *G. carbonaria* and *G.*

TABLE 3. MEAN ± STANDARD DEVIATION OF pH, VOLATILE FATTY ACID (VFA) CONCENTRATION (mM/LITER), ACETATE : PROPIONATE RATIO AND VFA MOLAR PERCENTAGES FOR FECES FROM *Gopherus polyphemus* FEEDING ON TWO DIETS. Sample size = number of fecal pellets (number of tortoises).

| | Aeschynomene | Mixed grazing |
|---|---|---|
| Sample size | 7 (5) | 7 (2) |
| pH | 8 ± 0 | 8 ± 0 |
| VFA concentration | 24.5 ± 7.7 | 23.1 ± 7.6 |
| Acetate : propionate | 13.8 ± 7.3 | 14.3 ± 2.2 |
| VFA molar % | | |
| Acetate | 81.8 ± 8.1 | 89.1 ± 1.5 |
| Propionate | 7.5 ± 3.6 | 6.2 ± 1.0 |
| i-Butyrate | 3.9 ± 1.5 | 1.6 ± 0.1 |
| n-Butyrate | 3.7 ± 3.0 | trace |
| i-Valerate | 3.0 ± 1.3 | 3.0 ± 0.4 |

*denticulata* feeding on both fruit and foliage diets (Bjorndal, in prep.). Clearly, potassium permanganate lignin should not be assumed to be an indigestible marker. As has been stressed elsewhere (Bjorndal, 1985b), markers should be tested for accuracy before they are used.

*Fecal pH and volatile fatty acids.*—Fresh feces were collected from tortoises feeding on either the high quality aeschynomene leaf diet or on a lower quality diet of grasses and forbs, and the pH, concentration of volatile fatty acids (VFA), acetate : propionate ratio, and molar percentages of VFA in the feces were measured (Table 3). VFA are the major end products of microbial fermentations in digestive tracts of herbivores and provide an important energy source to the host (Church, 1976).

Comparing these values with those of other herbivorous reptiles, pH is slightly higher but similar to that reported for feces of other species: 7.0 for *I. iguana* (Troyer, 1984b), 7.3 for *G. carbonaria* and 7.6 for *G. denticulata* (Bjorndal, in prep.), and 7.2 to 8.0 for *C. mydas* (Bjorndal, 1979, in prep.). The only data available for VFA concentrations in feces or rectum contents that are comparable in the method of collection and units of expression to the data presented here are from Guard (1980). He measured 12.7 mM of VFA per liter of feces for *I. iguana* and 9.1 mM/liter for *G. carbonaria*, but gave no information on diet. From a graph of VFA data in Troyer (1984b), it is evident that, as it is in gopher tortoises, acetate is the predominant VFA in the feces of *I. iguana*, with propionate and butyrate representing much smaller amounts.

Many species of herbivorous reptiles have either threatened or endangered survival status. Because of this, it is important to develop non-lethal methods with which the nutritional status of an animal or its diet quality can be quickly assessed in the field with little or no disturbance to the animal. One approach is to analyze feces for components that may be sensitive indicators. Because diet affects pH, VFA concentration, acetate : propionate ratio, and relative VFA molar percentages in ruminants (Church, 1976), these characteristics were tested for their potential as indicators of diet quality by comparing the values from feces of tortoises feeding on high and low quality diets.

There was no significant difference between pH, VFA concentrations, or acetate : propionate ratio in feces from the two diets ($P > 0.05$, Mann-Whitney U test), which suggests that they are not good indicators of diet quality. The biological or statistical significance of the differences between the patterns of VFA molar percentages is difficult to assess because of the inter-relatedness of the percentages and the compounds (Van Soest, 1982). The usefulness of these patterns for assessing diet quality needs to be evaluated.

*Conclusions.*—Because no vertebrate is known to produce cellulase, vertebrate herbivores must rely on a gut microflora to degrade the plant cell wall fraction of their diet. There are several basic requirements that are necessary to maintain an efficient gut microflora. Primary among these are constant, and preferrably elevated, body temperature; constant food supply; slow passage of digesta to allow sufficient time for microbial reproduction; anaerobic conditions; control of gut pH; and removal of fermentation waste products.

The fact that cellulose is degraded to a significant extent in tropical reptiles indicates that they are capable of meeting these requirements. Temperate herbivorous reptiles are under more severe environmental constraints, which would seem to make it more difficult for temperate reptiles to meet the requirements for maintaining an efficient cellulolytic microflora. Temperate regions are characterized by greater temperature fluctuations and long periods with limited food availability. However, it is clear from the high percentage of cellulose digested, that gopher tortoises maintain an efficient gut

microflora. The deep burrows in which gopher tortoises live buffer environmental temperature fluctuations, and thus help tortoises maintain a more constant body temperature. Whether reptiles maintain their gut bacteria during hibernation and/or aestivation (up to 5 mo for gopher tortoises), or whether they rely on reinoculation, needs further investigation.

The 13 d passage time measured in this study would provide ample time for microbes to attach to the digesta, feed and reproduce before being flushed from the digestive tract. Also, anaerobic conditions necessary for fermentation are met because there is no significant source of oxygen in the hindgut.

The high pH and relatively low VFA concentrations in the feces indicate that gopher tortoises are able to absorb the fermentation waste products—primarily VFA—and thus gain energy from the fermentation. The VFA from a hindgut fermentation can make a significant contribution to the energy balance of herbivorous reptiles. Bjorndal (1979) estimated that the fermentation in the cecum alone (not including the rest of the hindgut) provided 15% of the energy requirements of *C. mydas.* McBee and McBee (1982) estimated that 30–40% of the energy requirements of *I. iguana* was provided by the fermentation in its hindgut.

This study has demonstrated that at least one temperate herbivorous reptile can maintain a cellulolytic microflora that makes a significant contribution to its nutritional balance. The extent to which other temperate reptiles rely on gut microflora, and how the requirements of the microflora are met, requires further study.

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## Literature Cited

Bjorndal, K. A. 1979. Cellulose digestion and volatile fatty acid production in the green turtle, *Chelonia mydas.* Comp. Biochem. Physiol. 63A:127–133.

———. 1980. Nutrition and grazing behavior of the green turtle, *Chelonia mydas.* Mar. Biol. 56:147–154.

———. 1985a. Nutritional ecology of sea turtles. Copeia 1985:736–751.

———. 1985b. Use of ash as an indigestible dietary marker. Bull. Mar. Sci. 36:224–230.

Carr, A. F. 1952. Handbook of turtles. Cornell University Press, Ithaca, New York.

Christian, K. A., C. R. Tracy and W. P. Porter. 1984. Diet, digestion, and food preferences of Galapagos land iguanas. Herpetologica 40:205–212.

Church, D. C. 1976. Digestive physiology and nutrition of ruminants. Vol. 1. 2nd ed. O & B Books, Corvallis, Oregon.

Gallaher, R. N., C. O. Weldon and J. G. Futral. 1975. An aluminum block digester for plant and soil analysis. Soil Sci. Soc. Amer. Proc. 39:803–806.

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures and some applications). Agriculture Handbook No. 379. U.S. Dept. of Agric., Washington, D.C.

Golding, E. J., M. F. Carter and J. E. Moore. 1985. Modification of the neutral detergent fiber procedure for hays. J. Dairy Sci. 68:2732–2736.

Guard, C. L. 1980. The reptilian digestive system: general characteristics, p. 43–51. *In:* Comparative physiology: primitive mammals. K. Schmidt-Neilsen, L. Bolis, C. R. Taylor, P. J. Bentley and C. E. Stevens (eds.). Cambridge University Press, Cambridge, United Kingdom.

Hambleton, L. G. 1977. Semiautomated method for simultaneous determination of phosphorus, calcium and crude protein in animal feeds. J.A.O.A.C. 60:845–852.

Hamilton, J., and M. Coe. 1982. Feeding, digestion and assimilation of a population of giant tortoises (*Geochelone gigantea* (Schweigger)) on Aldabra atoll. J. Arid Envir. 5:127–144.

McBee, R. H., and V. H. McBee. 1982. The hindgut fermentation in the green iguana, *Iguana iguana,* p. 77–83. *In:* Iguanas of the world: their behavior, ecology and conservation. G. M. Burghardt and A. A. Rand (eds.). Noyes Publications, Park Ridge, New Jersey.

Nagy, K. A. 1977. Cellulose digestion and nutrient assimilation in *Sauromalus obesus,* a plant-eating lizard. Copeia 1977:355–362.

———, and P. A. Medica. 1986. Physiological ecology of desert tortoises in southern Nevada. Herpetologica 42:73–92.

Parmenter, R. R. 1981. Digestive turnover rates in freshwater turtles: the influence of temperature and body size. Comp. Biochem. Physiol. 70A:235–238.

Troyer, K. 1982. Transfer of fermentative microbes between generations in a herbivorous lizard. Science 216:540–542.

———. 1984a. Diet selection and digestion in *Iguana iguana*: the importance of age and nutrient requirements. Oecologia 61:201–207.

———. 1984b. Structure and function of the diges-

tive tract of a herbivorous lizard *Iguana iguana*. Physiol. Zool. 57:1–8.
VAN SOEST, P. J. 1982. Nutritional ecology of the ruminant. O & B Books, Corvallis, Oregon.
VOORHEES, M. E. 1981. Digestive efficiency of *Sauromalus varius*. Unpubl. M.S. thesis, Colorado State University, Fort Collins, Colorado.

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# Persistence of Freeze Tolerance in Terrestrially Hibernating Frogs after Spring Emergence

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**Freeze tolerance is an adaptation for winter survival in various species of terrestrially-hibernating frogs. We assessed the persistence of freeze tolerance in four species collected at breeding ponds after the spring emergence from hibernation. *Rana sylvatica, Hyla versicolor, H. crucifer,* and *Pseudacris triseriata* were as tolerant of whole body freezing in early spring as they were in autumn or winter, based on survival at −2.5 C for periods ranging up to 8 d. In later spring, after animals had begun to feed, tolerance to freezing declined sharply. Whole animal supercooling points (−1.2–−2.5 C) were the same in spring as in autumn but the capacity for producing cryoprotectants in response to the initiation of freezing was generally reduced in spring animals. Levels of glucose or glycerol produced in spring animals during freezing were often at least 10 fold lower than amounts which typically accumulate in animals in autumn or winter under equivalent freezing exposures. This reduced capacity for cryoprotectant synthesis may have resulted from lower rates of cryoprotectant synthesis in spring animals or the commitment of liver glycogen reserves to other uses in spring animals.**

FOR poikilothermic vertebrates hibernating on land, the subzero temperatures of winter seriously threaten survival. However, a number of terrestrially-hibernating amphibian species occur in the northern United States and southern Canada. Toads and salamanders supercool only slightly (to about −2 C) and are killed by internal freezing (Storey and Storey, 1986a). These animals avoid exposures to low subzero temperatures by behavioral means; for example, *Bufo americanus* digs about 1 m down into the soil (Froom, 1982).

A natural tolerance for the freezing of extracellular water has developed in four species of frogs: the wood frog, *Rana sylvatica,* the grey tree frog, *Hyla versicolor,* the spring peeper, *H. crucifer,* and the chorus frog, *Pseudacris triseriata* (Schmid, 1982; Storey, 1984a, 1985). These animals spend the winter at the soil surface hidden under leaves, logs, tree roots, or rocks (Froom, 1982) and covered with a blanket of snow; the microclimate of such a protected hibernation site is not often severe but temperatures of −5–−7 C occur (Schmid, 1982; MacArthur and Dandy, 1982). Since whole animal supercooling points are only −2–−3 C (Schmid, 1982; Storey, 1985; Storey and Storey, 1986a) animals often face one or more bouts of freezing in a winter. Freezing occurs in extracellular compartments producing large masses of ice in the abdominal cavity and abundant crystals under the skin all over the body. Ice contents of 35–48% of total body water are tolerated (Schmid, 1982; Storey, 1984a). When frozen, limbs are no longer extendible, and breathing, blood flow, and heart beat are temporarily suspended. Low molecular weight cryoprotectants are accumulated and used within cells to limit dehydration