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## The Use of Stable Isotopes to Determine the Physiology and Ecology of Reptiles

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### Introduction

Stable isotope research is becoming more widely used in physiological ecology. Atoms of the same atomic number but different atomic weights are called isotopes. Nitrogen (N) has two isotopes,  $^{14}\text{N}$  and  $^{15}\text{N}$ , with the latter being the heavier isotope. The assumption is that  $^{14}\text{N}$  is excreted at a higher rate than  $^{15}\text{N}$ . It is also thought that this should cause an increase in  $^{15}\text{N}$  of the protein tissues and preferential excretion of  $^{14}\text{N}$  of an animal when the animal is in the process of breaking down proteins.

Many of these assumptions are based on a study of eight cows (Steele 1977). This is problematic because “assimilation and metabolic pathways that lead to fractionation differ among animals” (Gannes *et al.* 1997). Cows and lizards use different metabolic pathways to excrete nitrogenous wastes. Cows excrete urea and lizards excrete uric acid, a white semi-solid suspension via the kidneys. It is therefore possible that the  $^{15}\text{N}/^{14}\text{N}$  ratios in the excrements of these two groups differ relative to their diets and the  $^{15}\text{N}/^{14}\text{N}$  ratio in the excrements may not differ significantly from the  $\delta^{15}\text{N}$  in the diet. We tested this hypothesis using side-blotched lizards (*Uta stansburiana*) (experiment #1).

Secondarily, a study suggests animals in dry climates maintain a higher  $^{15}\text{N}/^{14}\text{N}$  ratio in their tissues than similar animals in humid climates (Ambrose 1993). Uric acid formation requires only a small amount of water to create. Animals that excrete uric acid are usually adapted to conditions of limited water. We decided to test a hypothesis that water deprived animals show an increase in  $^{15}\text{N}/^{14}\text{N}$  within and across species based on the two findings above (experiment #2). We used two species of lizard: side-blotched lizards, an arid climate lizard, and American anoles (*Anolis carolinensis*), a lizard typically found in humid climates.

### Materials and Methods

In October 2002, we obtained 36 side-blotched lizards from St. George, Utah. They were divided equally among three large aquaria, and fed a diet of crickets *ad libitum*. In May 2003, we separated the lizards and broke off a piece of tail from each. We collected feces and uric acid over a period of two weeks. Feces, uric acid, tail tissue, and crickets were analyzed for nitrogen and carbon content using a Costech elemental analyzer and a Finnigan Delta II Plus mass spectrometer. Each sample weighed less than 2 mg and was placed in a small Costech tin capsule in preparation for analysis. To test whether  $^{14}\text{N}$  is preferentially excreted compared to  $^{15}\text{N}$ , we compared the  $\delta^{15}\text{N}$  of the diet (crickets) to the uric acid, feces, and tissue (tail).

In July 2003, we tested the hypothesis that water deprivation causes a fractionation in N isotopes using 6 side-blotched lizards and 7 American anoles. Both groups were fed *ad libitum* on a diet of crickets. For two weeks, the control group was given water *ad libitum* and the treatment group was not given water unless necessary for the animal's survival. Again, we measured the

$\delta^{15}\text{N}$  of the crickets, tail, uric acid, and feces using the same analytical procedure as the first experiment.

## Results

Experiment 1. The tail was  $^{15}\text{N}$  enriched compared to diet, but less than other studies. Both uric acid and feces were  $^{15}\text{N}$  depleted relative to diet. Carbon isotope values showed no significant difference from the diet.

Experiment 2. Water deprived animals show an increase in  $^{15}\text{N}/^{14}\text{N}$  ratio in the feces when compared to the controls. The  $^{15}\text{N}/^{14}\text{N}$  ratios of the uric acid of the lizards did not differ between the treatment and control groups. Carbon isotopes did not differ significantly in any area. The two different species of lizard differed in their  $^{15}\text{N}/^{14}\text{N}$  ratios. There was no difference in the reaction of the treatment between species

## Conclusions

Nitrogenous wastes appear to be depleted of  $^{15}\text{N}$  relative to diet across taxa. Our study supports the conclusion that body tissue is enriched in  $^{15}\text{N}$  relative to diet across taxa. Uric acid  $\delta^{15}\text{N}$  levels in animals deprived of water do not differ significantly from the  $\delta^{15}\text{N}$  in the uric acid of animals with an abundance of water. Our study suggests that the metabolic pathways of urea formation and uric acid formation both preferentially excrete  $^{14}\text{N}$ . More studies could be conducted using different species.

## References

- Ambrose, S. H., and L. Norr. 1993. Experimental evidence for the relationship of the carbon isotope ratios of the whole diet and dietary protein to those of one collagen and carbonate, p. 1-37. J.B. Lambert and G. Grupe [eds.]. In *Prehistoric human bone: archeology at the molecular level*. Springer-Verlag, Berlin.
- DeNiro, M.J., and S. Epstein. 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science*. 197:261-263.
- Gannes, L.Z., D. O'Brien, and C. Martinez del Rio. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. *Ecology*. 78:1271-1276.
- Hatch, K.A., B. Pinshow, and J.R. Speakman. 2002. Carbon isotope ratios in exhaled  $\text{CO}_2$  can be used to determine not just present, but also past diets. *J. Comp. Physiol. B*. 172:263-268.
- Magnusson, W.E., A.P. Lima, A.S. Faria, R.L. Victoria, and L. A. Luiz. 2001. Size and carbon acquisition in lizards from Amazonian savanna: Evidence from isotope analysis. *Ecology*. 6:1772-1780.
- Steele, K.W., and R.M. Daniel. 1977. Fractionation of nitrogen isotopes by animals: a further complication to the use of variations in the natural abundance of  $^{15}\text{N}$  for tracer studies.