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Comparative skin permeability of neonatal and adult timber rattlesnakes (*Crotalus horridus*)

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Abstract

Skin permeability and lipid content were determined using shed epidermis of neonatal and adult timber rattlesnakes (*Crotalus horridus*) from the Coastal Plain Pine Barrens of New Jersey and from the Appalachian Mountains of northern Pennsylvania. Differences between populations due to habitat and within populations due to age were tested. Skin permeability was not found to differ according to locality ($P > 0.05$), but rates were significantly different for age. Permeability of adult epidermis was greater than that of neonates ($P < 0.01$). Lipid content did not differ by locality ($P > 0.05$), but differed between ages, paralleling the results found for permeation rates. Neonate sheds had a greater amount of extractable lipids than adult sheds ($P < 0.01$). Despite the lower skin permeability of neonates, our estimates indicate that the percentage of their total body water content lost per hour may still be 2.2 times that of adults. Resistance to cutaneous water loss may be advantageous to neonates given their relatively large surface area-to-volume ratio.

Keywords: *Crotalus horridus*; Ecdysis; Evaporative water loss; Habitat; Lipids; Shed epidermis; Skin permeability; Snake

1. Introduction

Research beginning in the 1960s refuted the assumption that the reptilian integument is essentially impermeable to water (Bentley and Schmidt-Nielsen, 1966). Of the sources of evaporative water loss, cutaneous water loss seems to be the most significant for snakes (e.g., Prange and Schmidt-Nielsen, 1969; Dmi'el, 1972; Cohen, 1975). Lipids, located primarily in the mesos layer of the epidermis, have been shown to function as the main barrier to evaporative water loss (Roberts and Lillywhite, 1980, 1983). Evaporative water loss rates and skin permeability are affected by factors such as habitat aridity, exposed surface area, temperature, position of the animal in the shedding cycle, activity, and hydration state of the epidermis (Lillywhite and Maderson, 1982; Mautz, 1982; Winne et al., 2001). Although interspecific comparisons abound, little attention has been given to intraspecific differences in water loss, especially the effects of habitat variation and age on the water loss of conspecific snakes. The current study was designed to examine the occurrence of such intraspecific variation in the cutaneous water loss of timber rattlesnakes (*Crotalus horridus*).

We compared skin permeability and epidermal lipid content between two populations of *C. horridus* that occupy different habitats. In the Appalachian Mountains of Pennsylvania, *C. horridus* inhabits deciduous hardwood forest interspersed with open, rocky areas (Reinert, 1984a,b). In this region, overwintering hibernacula are typically associated with rock ledges and crevices on south-facing slopes (Martin, 1992; Brown, 1993). In the coastal plain of southern New Jersey, this species inhabits low-elevation forests dominated by pitch pine (*Pinus rigida*), and hibernacula are typically associated with wetland habitats where the snakes overwinter immersed in water (Burger, 1934; Kauffeld, 1957; Reinert and Zappalorti, 1988a,b).

We also compared skin permeability and lipid content of neonatal and adult *C. horridus* for these two populations.

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Because rattlesnakes bear live young (Klauber, 1972), neonates possess the skin they had while inside their mother. This neonatal integument is presumed to be more permeable than adult skin to allow passage of nutrients from the amniotic fluid (Graves et al., 1986). It has been suggested that the first ecdysis helps neonates to function in their new terrestrial environment through the sloughing of this more permeable "proto-integument" (Graves et al., 1986; Tu et al., 2002). Likewise, the aggregative behavior observed in many rattlesnake species (including *C. horridus*) prior to the first ecdysis is often viewed as a direct effort by neonates to reduce water loss (Graves et al., 1986; Tu et al., 2002).

2. Materials and methods

2.1. Skin permeability

An in vitro preparation of shed outer epidermis was used to compare skin permeability from four experimental groups of *C. horridus* defined by age and location. Shed skins were obtained from five neonatal (first shed) and five adult *C. horridus* from the Coastal Plain Pine Barrens of southern New Jersey (Burlington Co. and Ocean Co.) and five neonatal (first shed) and five adult *C. horridus* from the Appalachian Mountains of northern Pennsylvania (Clinton Co., Lycoming Co., and Tioga Co.). Three samples were taken from the dorsal epidermis of each shed skin and soaked briefly in deionized water for ease of manipulation. The skin samples were gently stretched over 10×75 mm culture tubes (tube opening = 0.58 cm^2) containing 2.0 mL of deionized water with the inner surface of the epidermis facing the inside of the tube. Samples were held in place with thread, trimmed to remove excess skin around the neck of the tube, and then sealed with Parafilm® (American National Can, Greenwich, CT, USA), ensuring that the skin surface over the tube opening remained unobstructed. No skin other than the surface over the tube opening was exposed. The outer epidermal surfaces were examined under a stereomicroscope for tears, and damaged samples were discarded. The completed tubes were placed in an upright position overnight to allow the skin samples to achieve similar states of hydration. The tubes were then inverted, allowing the water to contact the inner surface of the skin sample to simulate in vivo conditions (Burken et al., 1985). Each inverted tube was suspended in the mouth of a 30 mL serum bottle containing 3.50 g of t.h.e.® desiccant (EMD Chemicals Inc., Gibbstown, NJ, USA) to remove moisture and maintain comparable humidity levels for each sample. Parafilm® was wrapped around the outer surface of each test tube to create a "stopper" to seal the system while allowing the test tubes to be removed for periodic measurements. Fig. 1 illustrates the in vitro preparation used to measure water permeation rates. Sample tubes were weighed to the nearest 0.001 g at 12 h intervals. A total of 10 mass measurements were taken per sample. Leaking samples, noted by accumulation of water on the outer skin surface and/or substantial weight loss, were discarded. Discarded samples were replaced to maintain three skin samples per snake. The desiccant was replaced as necessary. Ambient laboratory temperature recorded at the time of each mass measurement ranged from 21.5 to 24.0 °C. All Pine Barrens samples were tested together, followed by all Appalachian Mountain samples.

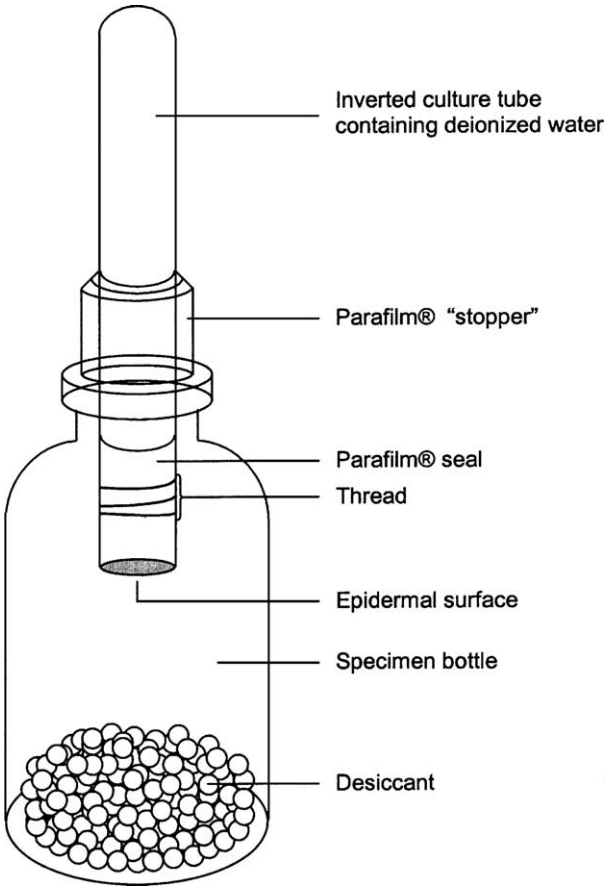

Fig. 1. Diagram of in vitro preparation of shed dorsal epidermis used to determine water permeation rates of neonatal and adult *Crotalus horridus* (see Materials and methods).

2.2. Lipid extraction

Lipid extraction was also performed on shed dorsal epidermis of five neonatal and five adult *C. horridus* from the two localities. The procedure used followed that of Stokes and Dunson (1982). Two samples per adult and two samples per neonate were used. Samples were first dried over 18.0 g t.h.e.® desiccant in sealed 250 mL beakers. After 24 h, initial masses were taken (to the nearest 0.001g). Samples were soaked for 24 h in a 2:1 chloroform/methanol (by volume) extraction mixture in sealed 175 cc specimen bottles. After another 24 h, the samples were removed from the mixture and sequentially washed once with ~10 mL of fresh 2:1 chloroform/methanol and twice with ~10 mL of