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Stability of Venoms from the Northern Pacific Rattlesnake (*Crotalus viridis oreganus*)

Abstract

Stability of proteins in venom samples extracted from twelve northern Pacific rattlesnakes (*Crotalus viridis oreganus*) over an eight month period (April-November, 1984) was demonstrated by SDS-polyacrylamide electrophoresis. The electrophoretic pattern of the venoms varied considerably between individual *C. v. oreganus*, but for the same individual remained identical from the time the rattlesnakes emerged from hibernation through their active, summer period. The electrophoretic variation of individual venoms did not appear to be a result of differences in geographic location or sex. Electrophoresis of the venoms revealed 8-14 protein components that ranged in molecular weight from approximately 6,000 to 66,000. The stability of the venoms suggests that the secretory epithelium of the venom glands maintain a consistent composition of proteins in the venoms. Thus, the effects of hibernation on venom composition are apparently negligible. Consequently, as *C. v. oreganus* emerge from hibernation they most likely maintain undiminished feeding and defensive capabilities.

Introduction

The northern Pacific rattlesnake (*Crotalus viridis oreganus*) is a subspecies of the western rattlesnake distributed in the temperate regions of the Pacific northwest, including northern California, Oregon, Washington, and parts of Idaho and British Columbia (Nussbaum *et al.* 1983). The *C. v. oreganus* from southeastern Washington are often subjected to extreme variations in climate due to seasonal changes. Environmental conditions range from hot, dry periods with temperatures exceeding 38°C during summer months (June-August) to prolonged periods of subfreezing temperatures in the winter (December-January). The rattlesnakes inhabiting this region survive the harsh winter conditions by hibernating for extended periods below the frost line in protected dens located throughout the area.

C. v. oreganus resume hunting soon after emergence from hibernation and continue throughout their active summer period. However, the effects that hibernation and seasonal changes have on the composition of *C. v. oreganus* venoms are not known. If the composition of venom changes during winter months, as in some snake species (Gubensek *et al.* 1974), then resumption of predatory and defensive behavior in the spring could be impaired.

In other species of venomous snakes, different species show different responses to seasonal change. Gubensek *et al.* (1974) have shown a difference in the electrophoretic migration of proteins in European long-nosed viper (*Vipera ammodytes*) venoms extracted during the winter months. Sket *et al.* (1973) also isolated two toxic proteins in the venoms from *V. ammodytes* that were absent in winter venom extractions. However, no change in venom composition occurred in jumping viper (*Bothrops nummifera*) venoms or in venoms from the African puff adder (*Bitis arietans*) regardless of the time of year the venoms were extracted (Jimmenz-Porras 1964, Willemse 1979). The purpose of the present investigation was to study the electrophoretic migration of venom proteins from newly captured *C. v. oreganus* collected early in the spring as they emerged from hibernation and compare the pattern to venoms extracted from the same individuals seven months later in the fall at the time of their normal return to hibernation to determine if any change occurs in the composition of their venoms.

Study Area and Methods

Two den sites located in Whitman and Lincoln counties of southeastern Washington State were checked regularly and northern Pacific rattlesnakes (*C. v. oreganus*) of both sexes collected early in April, 1984 as they emerged from hibernation. The rattlesnakes were sexed, measured, and housed in glass aquaria in an environmentally controlled room (25° + C; 12 hr. light/dark). The sizes of the *C. v. oreganus* used were: less than 50 cm (n = 2), 50-80 cm (n = 7), and greater than 80 cm (n = 3). The rattlesnakes were fed white mice of appropriate size approximately once a month throughout the investigation.

The *C. v. oreganus* were subjected to venom extraction within 24 hours of capture and then again seven months later in November, 1984. Venom samples from a total of 12 *C. v. oreganus* were individually collected into separate 1.5 ml plastic vials. The venoms were extracted by allowing each rattlesnake to bite naturally (no pressure applied to the venom glands) through a parafilm membrane stretched tightly over the opening of a glass funnel that emptied into the vials. The venoms were immediately frozen and then lyophilized at $-50°C$ for 24 hours. The vials containing lyophilized venoms were stored over dessicant at $-4°C$ in glass containers.

Electrophoresis

SDS-polyacrylamide electrophoresis (1.5 mm slab gel) was performed following Laemmli (1970) using a 4% stacking and 9% separating gel. The lyophilized venoms were reconstituted in sample buffer (pH 6.8) to a concentration of 2 mg/ml. Venoms extracted from the *C. v. oreganus* in April, 1984, and then again in November, 1984, were loaded into consecutive wells at a concentration of 0.08 mg venom per well. The electrophoresis (continuous current) was run at 5 mA/gel for 90 minutes followed by 30 mA/gel for 3 hours.

Upon completion of the electrophoresis, the gels were immediately stained in 0.001% Commassie Brilliant Blue (Sigma Chemical Co., Inc.) for 2-4 hours and destained using a 7% acetic acid, 35% methanol, and 58% distilled water solution. SDS-Dalton Mark VII-L markers (Sigma, Inc.) were used for approximate molecular weight determinations.

Results

The results of SDS-polyacrylamide electrophoresis of *C. v. oreganus* venoms extracted from three of the twelve individuals during the spring and fall, 1984, are shown in Figure 1. All twelve *C. v. oreganus* showed considerable individual differences in the protein components in their venoms, however, the composition of venom from the same snake did not change from April, 1984 through November, 1984.

SDS-polyacrylamide electrophoresis of crude venoms reduced with 2-mercaptoethanol revealed 8-14 protein components that ranged in molecular weight from approximately 6,000 to 66,000. The venoms contained two protein bands with molecular weights of approximately 30,000 and 60,0000 and two low molecular components of approximately 6,000 and 10,000 that appeared as dark staining bands in the gels and seemed to be present in all rattlesnake venoms used in the investigation. The remaining protein components in the venoms were not common to all rattlesnakes. The variation in electrophoretic pattern of protein components between individuals did not appear to correlate with the geographic location or the sex of the rattlesnakes.

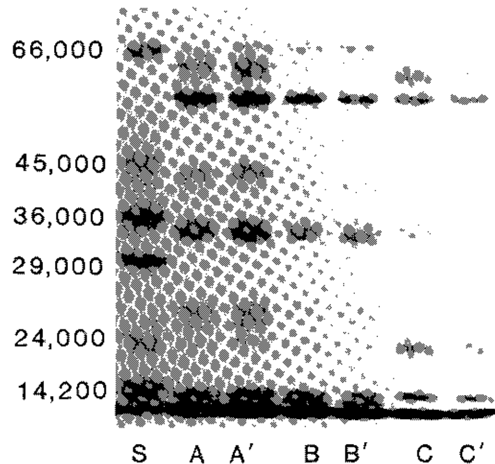

Figure 1. 9.0% SDS-polyacrylamide electrophoresis of *C. v. oreganus* venoms. Column S, molecular weight markers. Columns A, B, and C, venoms from three *C. v. oreganus* extracted during April, 1984. Columns A', B', and C', corresponding venoms from the same three rattlesnakes extracted seven months later.

Discussion

Numerous studies on snake venoms have demonstrated ontogenetic changes in the composition of venom proteins (Minton 1967, Fiero *et al.* 1972, Meirer 1986, Bonilla *et al.* 1973, Mackessy 1985). Geographic differences in the electrophoretic migration of venom proteins have also been shown in *Crotalus atrox* and *Bothrops asper* (Minton and Weinstein 1986, Aragon-Ortiz and Gubensek 1979). Furthermore, Gubensek *et al.* (1974) showed a seasonal variation in venoms from *V. ammodytes* even though the snakes were maintained in a controlled environment. The composition of venoms from other snake species, however, remained stable throughout the year (Jimenz-Porras 1964, Willemse 1979).

The venoms from captive *C. v. oreganus* maintained in a controlled environment over several years did not show any seasonal variation in the composition of proteins (unpublished results). SDS-polyacrylamide electrophoresis performed on the venoms from newly captured *C. v. oreganus* used in this experiment also revealed a consistency in venom proteins from the time the rattlesnakes emerged from hibernation (April) through their active summer period (May-October). The electrophoretic migration of venom proteins was observed for only one summer period to minimize the possibility of ontogenetic changes in composition.

The *C. v. oreganus* used in this experiment were maintained in a controlled environment that did not exactly duplicate natural environmental conditions. The rattlesnakes, however, were collected at a time when they were normally becoming active after a long period of hibernation. If hibernation had affected venom composition, then the electrophoretic migration of venoms collected as the snakes emerged in the spring should have been different from samples extracted after the snakes had been active throughout the summer, even though they were housed in a controlled environment. Since the venoms did not change, it seems likely that the effects of hibernation, if any, are very slight.

The stability of *C. v. oreganus* venoms implies that the secretory epithelium of the venom glands maintains a consistent composition and thus maintains the feeding and defensive capabilities of the snakes as they emerge from hibernation in the spring. Willemse (1979) proposed that the length of time the venom remains in the lumen of the venom glands could ultimately cause changes in the electrophoretic migration of proteins. Crotalid and viperid snake venoms normally contain numerous proteolytic enzymes (Bjarnason and Tu 1978, Kocholaty *et al.* 1971, Kurecki *et al.* 1978) that could produce peptide artifacts in the venoms through auto-cleavage of the proteins. The *C. v. oreganus* used in this investigation were feeding regularly and the venoms were not stored in the glands for identical periods between extractions. The electrophoretic pattern of the venoms from individual rattlesnakes, however, was identical in both spring and fall venom extractions. The length of time the venom remains in the glands of *C. v. oreganus* thus does not appear to be a significant factor determining the electrophoretic migration of venom proteins.

Intrasubspecific differences in the composition of individual snake venoms have been well documented (Meirer 1986). The *C. v. oreganus* from southeastern Washington also showed considerable individual variation in the electrophoretic migration of proteins in their venoms (Figure 1). The high degree of variation in protein components required that the venoms from individual *C. v. oreganus* be examined to prevent the possibility of intrasubspecific differences in pooled samples being interpreted as a change in composition.

It was not possible to sample venom from *C. v. oreganus* during actual hibernation with the experimental procedure used in this investigation. An undetected change in composition could have occurred but is rapidly stabilized once the rattlesnakes start to emerge in the spring. However, the present study suggests that *C. v. oreganus* in southeastern Washington demonstrate a stable electrophoretic migration of proteins in their venoms as they emerge from hibernation and throughout their active, summer period (April-October).

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