Variability of Venom–Neutralizing Properties of Serum from Snakes of the Colubrid Genus Lampropeltis

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Variability of Venom-neutralizing Properties of Serum from Snakes of the Colubrid Genus *Lampropeltis*

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**ABSTRACT.**—Venom neutralization properties and protein content of serum from 11 taxa of *Lampropeltis* were studied. Most serum samples contained 6.5% to 9.5% protein. *Lampropeltis g. getulus* and *L. g. floridana* serum showed the broadest spectrum of effective neutralization of venoms from 10 crotaline taxa. *Lampropeltis t. triangulum*, *L. t. hondurensis*, *L. mexicana greeri*, and *L. alterna* effectively neutralized many of the venoms assayed, but were less efficacious than the other *Lampropeltis* species tested. Most of the serum samples investigated had variably effective neutralization capacities for venoms with strong hemorrhagic activities (*Crotalus atrox*, *C. adamanteus*, *C. v. viridis*). Sera from *L. g. holbrooki* and *L. g. floridana* were particularly effective in neutralizing venoms of *Agkistrodon piscivorus conanti* and *A. contortrix mokasen*. Only *L. g. getulus*, *L. g. floridana*, and *L. ruthveni* sera neutralized over 100 LD_{50} of *C. v. helleri* venom per ml. Only four serum samples (*L. g. getulus*, *L. g. floridana*, *L. calligaster*, and *L. t. triangulum*) were effective against type A *C. s. scutulatus* venom (contained high concentrations of the potent neurotoxin, Mojave toxin). All *Lampropeltis* sera assayed had effective neutralization potential for type B *C. s. scutulatus* venom, which has strong hemorrhagic and proteolytic activities and lacks Mojave toxin. All serum samples assayed were ineffective against venom of the elapid *Micrurus f. fulvius*. Serum from *Elaphe g. guttata* effectively neutralized several crotaline venoms, while *Rhinocheilus lecontei antonii* serum had only marginal neutralization capacity for several venoms. Serum from *Pituophis melanoleucus sayi* and the natricine *Thamnophis s. sirtalis* had no neutralization capacity for any venom tested. Venom-neutralizing serum proteins of *Lampropeltis* appear to be most effective against hemorrhagic and proteolytic venoms, with little or no neutralization capacities against venoms containing high concentrations of hypotensive peptides, post- or presynaptically acting neurotoxins, and/or myolytic phospholipases A_{2}.

Venom-neutralizing properties of ophidian serum have been noted by researchers since the mid-eighteenth century. Numerous investigators have reported autoimmunity of venomous species to their own venoms (Fontana, 1787; Kellaway, 1931; Philpot and Deutsch, 1956; Clark and Voris, 1969; Philpot et al. 1978; Weinstein et al., 1991). Other workers have noted the venom-neutralizing capacities of serum from both ophiphagic and non-ophiphagic, aglyphic, or opisthodegadont colubrids (Philpot and Smith, 1950; Bonnett and Guttman, 1971; Philpot et al., 1978; Lomonte et al., 1982; Nahas et al., 1983; Tomihara et al., 1988). Thus, while attention has been given to venom neutralization properties of serum from individual ophidian species, little attempt has been made to determine the spectrum of venom neutralization capacities of serum from different species of a given genus. Some members of the genus *Lampropeltis* fill a prominent ophiphagous niche, from North America to northwestern South America, and possess serum immunity against crotaline viperid venoms. We therefore compared venom
neutralization properties of serum from 11 taxa of *North American crotaline venoms*.

**Materials and Methods**

**Sources of Sera and Venoms.**—Captive-born (second or third generation) specimens of *Lampropeltis g. getulus* (two specimens, one male, one female), *L. g. californiae* (banded and striped phases, one specimen of each, female and male, respectively), *L. g. floridana* (two specimens, both males), *L. g. holbrooki* (one specimen, male), *L. g. splendida* (one specimen, female), *L. alternata* (one specimen, male), *L. triangulum hondurensis* (two specimens, both males), *Pituophis melanoleucus sayi* (one specimen, male), and *Elaphe g. guttata* (two specimens, one male, one female) were maintained on a weekly diet of either weanling or adult Swiss-Webster mice. Collected specimens were as follows: *L. t. triangulum* (three specimens, one male, two female, vicinity of Albany, New York) and *Thamnophis s. sirtalis* (four specimens, two male, two female, vicinity of Albany, New York), *Crotalus s. scutulatus* (one specimen, male, venom type A, Yuma, Arizona), *C. atrox* (>200 specimens, Archer County, Texas), *C. adamanteus* (one specimen, male, Tampa, Florida), *Agkistrodon piscivorus conanti* (one specimen, male, Tampa, Florida), and *A. contortrix mokasen* (three specimens, two females, one male, Frederick County, Maryland). A specimen of *Rhinocheilus lecontei antonii* (female) was from an unknown locality in Mexico. An additional specimen (male) of *C. s. scutulatus* (venom type B) and specimens of *C. horridus horridus* (two males) were of unknown provenience. The wild-caught specimens were maintained on the same diet as the captive-born specimens. However, all specimens of each species. However, all of the snakes were exposed to a 12 h light/dark cycle.

Blood samples were obtained by cardiac puncture with a 26½ gauge needle. Snakes were fasted 2 to 3 weeks prior to drawing of blood samples and anesthetized with ketamine hydrochloride (50 mg/kg, intramuscularly). Freshly drawn samples were allowed to clot overnight at 4 C, centrifuged at 7500 rpm, and the serum was removed with a pipette. Any sample not used immediately was frozen at −25 C. Serum samples were not heated prior to assay.

Venom samples were extracted manually every 3 to 4 weeks, frozen immediately at −25 C and lyophilized. Lyophilized venoms were stored in the dark at 4 C, over desiccant. Lyophilized pooled venoms of *C. viridis viridis* (Texas), *C. v. helleri* (California), *Sistrurus miliarius barbouri* (Florida), and *M. f. fulvius* (Florida) were purchased from Biotoxins Inc. (St. Cloud, Florida).

**Lethal Potency Determinations.**—Venom doses were derived from 1 mg of venom per ml of phosphate buffered saline (PBS, pH 7.2). Intraperitoneal (i.p.) LD₅₀ were obtained by injecting male Swiss-Webster mice (18–20 g) in three to five groups of four mice each. Animals were observed after injection and mortality recorded after 24 h. Animals succumbing to injections were necropsied and any gross tissue pathology noted. The LD₅₀ was calculated by the Spearman-Karber method (World Health Organization, 1981). The fiducial (95% confidence) limits for the LD₅₀ were determined.

**Estimation of Protein Content.**—Protein content was determined using the bicinchoninic acid assay (BCA assay, Pierce Chemicals, Rockford, Illinois; Smith et al., 1983).

**Venom Neutralization Assays.**—Venom solutions that contained 4.0 to 21.0 LD₅₀ were mixed with 3.0 mg of ophidian serum protein. These mixtures contained material sufficient for injection of four mice. Serum concentrations were standardized with the BCA assay and 750 μg of serum protein was used per venom dose. The serum-venom mixtures were incubated at 37 C for 45 min, then injected i.p. into mice. Several control animals were injected with serum alone or with normal rabbit serum. Injected animals were observed for 24 h, and any mortalities recorded and necropsied. Values representing neutralization potential of a given serum sample are expressed as the number of LD₅₀ completely neutralized (resulting in 100% survival) by 1 ml of serum. These data were calculated by dividing the total serum protein content (mg protein/ml serum) by the neutralizing serum protein sample (750 μg or 1 mg). This quotient was then multiplied by the number of LD₅₀ neutralized by the 750 μg or 1 mg serum protein sample. Due to the large numbers of mice required for these experiments, only a single determination at each dose level was possible. However, doses producing 100% survival were repeated at least once. In addition, the same constraint prevented investigating different specimens of each species. However, all specimens procured in pairs or trios were examined separately prior to pooling of serum samples.

**Results**

**Lethal Potencies of Selected Crotaline and Micrurine Venoms.**—Table 1 shows the murine i.p. LD₅₀ values determined for the crotaline and micrurine species selected for neutralization studies. Mice injected with lethal doses of most of the
### Table 1. Lethal potencies of venoms used in neutralization studies. Abbreviations: A = range of eight individual venoms from specimens collected in southwestern Utah; B = values were obtained for *A. piscivorus piscivorus* venom.

<table>
<thead>
<tr>
<th>Species (locality)</th>
<th>i.p. lethality (murine LD$_{50}$, mg/kg) [95% confidence limits]</th>
<th>i.p. LD$<em>{50}$ values reported previously (murine LD$</em>{50}$, mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Crotalus adamanteus</em> (Tampa, Florida)</td>
<td>3.30 [2.92-3.74]</td>
<td>1.89, $^1$ 3.75, $^7$ 2.7$^9$</td>
</tr>
<tr>
<td><em>C. atrox</em> (Archer Co., Texas)</td>
<td>4.79 [4.07-5.64]</td>
<td>6.0, $^4$ 8.42, $^3$ 4.5$^5$</td>
</tr>
<tr>
<td><em>C. viridis viridis</em> (Texas)</td>
<td>2.37 [2.09-2.70]</td>
<td>2.25, $^4$ 2.0, $^7$ 2.25$^9$</td>
</tr>
<tr>
<td><em>C. v. helleri</em> (California)</td>
<td>1.84 [1.53-2.20]</td>
<td>1.56, $^6$ 2.44, $^7$ 2.8$^9$</td>
</tr>
<tr>
<td><em>C. scutulatus scutulatus</em> (type A, Yuma, Arizona)</td>
<td>0.17 [0.143-0.199]</td>
<td>0.18, $^7$ 0.23, $^6$ 0.13-0.54$^{13}$</td>
</tr>
<tr>
<td><em>C. s. scutulatus</em> (type B, unknown)</td>
<td>2.72 [2.29-3.23]</td>
<td>2.3-3.8$^3$</td>
</tr>
<tr>
<td><em>C. horridus horridus</em> (unknown)</td>
<td>3.31 [2.89-3.80]</td>
<td>2.91, $^6$ 2.69$^{10}$</td>
</tr>
<tr>
<td><em>Sistrurus m. barbouri</em> (Florida)</td>
<td>9.40 [8.59-10.29]</td>
<td>6.0, $^4$ 6.84$^9$</td>
</tr>
<tr>
<td><em>Agkistrodon contortrix mokasen</em> (Frederick Co., Maryland)</td>
<td>15.06 [13.57-16.71]</td>
<td>7.8, $^4$ 8.9, $^7$ 10.50$^9$</td>
</tr>
<tr>
<td><em>A. piscivorus conanti</em> (Tampa, Florida)</td>
<td>5.33 [4.88-5.82]</td>
<td>5.10, $^5$ 5.11$^{11}$</td>
</tr>
<tr>
<td><em>Micrurus f. fulvius</em> (Florida)</td>
<td>0.62 [0.53-0.73]</td>
<td>0.67, $^7$ 0.9$^{13}$</td>
</tr>
</tbody>
</table>

$^1$ Russell and Emery (1959); $^2$ Hall and Gennaro (1961); $^3$ Glenn and Straight (1978); $^4$ Githens and Wolff (1939); $^5$ Minton (1956); $^6$ Russell (1967); $^7$ Kocholaty et al. (1971); $^8$ Minton (1974); $^9$ Glenn and Straight (1977); $^{10}$ Russell (1980); $^{11}$ Gingrich and Hohenadel (1956); $^{12}$ Cohen et al. (1971).

Crotaline venoms studied showed distress within 1–2 h post-administration. Venoms of *C. atrox, C. adamanteus, C. viridis viridis, S. miliarius barbouri,* *A. contortrix mokasen,* and *A. piscivorus conanti* caused brief periods of hyperactivity, which led to lethargy, prostration, and cardiorespiratory death. Necropsy of animals succumbing to effects of any of these venoms revealed marked hemorrhage at the injection site and varying degrees of mesenteric hemorrhage accompanied by extravasation of sero-sanguinous fluid. Venom samples of *A. c. mokasen* and *S. m. barbouri* were considerably less toxic than those reported previously (Table 1). Venoms from the two different populations of *C. scutulatus* produced vastly different effects. Populations of *C. s. scutulatus* that secrete venom containing the lethal, presynaptically-acting, polypeptide neurotoxin, Mojave toxin, are termed venom type A, while other populations that do not have this venom component are designated type B (Glenn and Straight, 1978). *Crotalus s. scutulatus* type A venom caused rapid prostration accompanied by tachypnea, “in-pouching” of the flank, and rapid cardiorespiratory death. Necropsy was unremarkable, except for minor pulmonary congestion. Administration of *C. s. scutulatus* type B venom produced effects very similar to those described for the majority of the aforementioned crotaline venoms. Significant hemorrhage and extravasation were observed upon necropsy. *Crotalus horridus* and *C. v. helleri* venoms produced lethal effects more rapid in development than those of the other crotalines, except for that of *C. s. scutulatus* (type A). *Crotalus horridus* venom caused rapid prostration, tachypnea, and extensive hemorrhage. Necropsy showed copious sero-sanguinous fluid in the peritoneal cavity and mild pulmonary hemorrhage. Administration of *C. v. helleri* venom produced rapid collapse and death accompanied by hemorrhage at the injection site and pulmonary petechiae.

Venom from the micrurine elapid, *M. f. fulvius,* produced tachypnea, prostration, collapse, and cardiorespiratory death. This clinical course proceeded more rapidly than most of the crotaline venoms investigated. Mice injected with
0.5 to 1.0 LD₅₀ excreted dark urine within 8 h of administration. Examination of the urine by ultrafiltration (Kellner and Alexander, 1986) demonstrated that the mice were producing copious myoglobinuria.

Protein Content of Sera.—Table 2 shows the protein content of ophidian serum samples selected for study. Examination of serum samples from several individuals of a given species (L. g. getulus, L. t. triangulum, L. m. greeri, T. s. sirtalis) indicated that reproducibility of protein content was within 10%. Serum samples from L. g. getulus, L. g. hoibrooki, L. g. floridana, L. g. californiae (striped phase), L. ruthveni, L. alterna, and L. calligaster consistently contained >65 mg protein per ml while samples from L. m. greeri and L. t. triangulum had 60 to 65 mg protein per ml (Table 2). Lampropeltis g. splendida and L. g. californiae (banded phase) samples had lower protein levels (52 and 54 mg/ml, respectively). Serum samples from Elaphe g. guttata and Pituophis melanoleucus sayi had protein concentrations of 66 and 80 mg/ml, respectively. Serum from Rhinocheilus lecontei antonii and L. t. hondurensis had the highest and lowest protein concentrations (120 mg/ml and 47 mg/ml), respectively. Serum from specimens of L. g. floridana consistently showed hyperlipemia greater than that observed with the other serum samples.

Venom-neutralization Capacities of Sera from Lampropeltis, Elaphe, Pituophis, Thamnophis and Rhinocheilus.—Table 2 shows the neutralization capacities of serum from selected species of Lampropeltis.

Lampropeltis g. getulus.—Of the taxa investigated, serum from the nominate race of L. g. getulus had the widest spectrum of protection against the venoms tested in neutralization assay. Serum samples from two specimens had almost identical neutralization potentials (within 7% of each other) for the venoms tested. Compared with the other ophidian sera investigated, L. g. getulus serum neutralized the most LD₅₀ of the venoms tested, except for those of S. m. barbouri, A. p. conanti, and A. c. mokasen. Of all the sera tested, L. g. getulus serum had the least effective neutralizing capacity for A. c. mokasen venom.

Lampropeltis g. floridana.—This serum showed a broad spectrum of protection similar to that of L. g. getulus (Table 2).

Lampropeltis g. splendida.—This serum consistently exhibited a relatively low neutralization potential for most of the venoms tested (Table 2). Although neutralization potential for A. c. mokasen venom was equal to that of L. ruthveni and very similar to that of L. g. californiae (banded phase) and L. t. triangulum, this serum had...
Lampropeltis g. californiae (striped phase vs. banded phase).—Serum from these two phases of L. g. californiae exhibited markedly different venom neutralization properties (Table 2). Even though both phases showed a broad spectrum of protection, serum from the striped phase had greater neutralization capacity for most venoms assayed (Table 2). The banded phase had neutralization potential for C. v. helleri venom, while serum from the striped form had no such capacity (Table 2). Serum from the striped phase neutralized S. m. barbouri venom more efficiently than any other serum tested (Table 2).

Lampropeltis g. holbrooki.—Serum from this species provided particularly effective protection against venoms of C. v. viridis, C. h. horridus, and A. c. mokasen, and A. c. conanti (Table 2). Of all the serum samples tested, L. g. holbrooki and L. g. floridana serum had the highest neutralization capacities for A. p. conanti venom, and showed equal neutralization potentials for this venom (Table 2). Serum from L. g. holbrooki and L. g. californiæ (banded phase) had the lowest neutralization potentials for C. s. scutulatus (type B) venom (Table 2).

Lampropeltis calligaster.—This serum was effective in neutralizing C. s. scutulatus venom type A, although the neutralization potential for this venom was about 25% less than that of L. g. getulus serum. Effective neutralizing capacity against C. s. scutulatus venom type B was also noted (Table 2).

Lampropeltis m. greeri.—Serum from this race of L. mexicana had relatively low neutralization potential for most of the venoms assayed (Table 2).

Lampropeltis ruthveni.—This serum was one of only three samples that neutralized over 100 LD₅₀ C. v. helleri venom per ml of serum (Table 2).

Lampropeltis alterna.—This serum was the only sample assayed that had relatively moderate neutralizing potential for C. atrox venom, but had no capacity to neutralize C. adamanteus venom (Table 2).

Lampropeltis t. triangulum.—In common with L. g. splendida and L. alterna, serum from L. t. triangulum had about 40% less neutralization potential for C. adamanteus venom than that of C. atrox (Table 2).

Lampropeltis t. hondurensis.—Of the samples tested, this serum had the lowest neutralization potential for C. atrox and C. v. viridis venoms (Table 2). Nominate L. triangulum serum had about 70% greater neutralization potential than that of L. t. hondurensis for C. atrox venom.

Rhinocheilus l. antonii.—Serum from Rhinocheilus marginally protected animals injected with venoms of S. m. barbouri, A. p. conanti, or A. c. mokasen (1.0 LD₅₀ of each of the venoms administered with 750 μg serum protein resulted in one mortality in a group of four mice).

Elaphe g. guttata.—Serum from this species effectively neutralized A. c. mokasen, A. p. conanti, and C. v. viridis venoms (132, 154 and 132 venom LD₅₀/ml of serum, respectively).

Pituophis m. sayi and T. s. sirtalis.—Serum from either species had no neutralization potential against any of the venoms tested.

Observations from Neutralization Assays (Crotalus atrox, C. adamanteus, C. v. viridis, C. h. horridus, C. s. scutulatus (type B), S. m. barbouri, A. c. mokasen, A. p. conanti venoms).—Mice injected with serum protein (750 μg–1 mg) from Lampropeltis sp. that had marked neutralization capacity for these particular venoms (Table 2), mixed with venom doses at the limit of neutralization capacity of each respective serum, exhibited only transient lethargy with no other signs of envenomation. Several mice that survived injection with mixtures of the various serum samples and venoms of C. atrox, S. m. barbouri, or A. c. mokasen showed persistent inflammation and/or edema.

Crotalus v. helleri and C. s. scutulatus (type A) venoms.—Animals injected with serum samples that showed neutralization potential for these venoms, and venom doses at the limit of neutralization capacity, exhibited transient prosstration and respiratory distress. Full recovery was evident within 2–3 h. Only four serum samples (L. g. getulus, L. g. floridana, L. calligaster, and L. t. triangulum) demonstrated neutralization potential for type A C. s. scutulatus venom, while all samples except that of banded phase L. g. californiæ, had strong neutralization potential for type B C. s. scutulatus venom (Table 2).

Micrurus f. fulvius venom.—None of the serum samples investigated exhibited any neutralization potential for M. f. fulvius venom (Table 2).

**DISCUSSION**

Venom-neutralization capacities of Lampropeltis are well known from scientific literature and anecdotal description. Ditmars (1910, 1937) described minor local effects in a specimen of L. getulus bitten repeatedly by a much larger A. piscivorus. He also observed that injection of Lampropeltis specimens with venoms from crotalines failed to produce any lethal effects, while administration of venoms from elapids, such as Micrurus and Naja, resulted in rapid death. Philpot and Smith (1950) reported only minor edema in a L. getulus ssp. injected intramuscularly with 320 mg of A. piscivorus venom in a single injection, and over a gram of this venom in five
Studies of venom-neutralization properties of Lampropeltis demonstrate that serum from these ophidians can inhibit venom hemorrhagic activity (Rosenfeld and Glass, 1940) and proteolysis, thrombin and C. adamanteus venom-catalyzed clotting of fibrinogen and fibrinolysis by plasmin (Philpot and Deutsch, 1956; Philpot et al., 1978). Several investigations have demonstrated serum-mediated neutralization of the lethal effects of venom in mice (Philpot and Smith, 1950; Bonnett and Gutman, 1971; Philpot et al., 1978). Philpot and Deutsch (1956) reported also that L. g. floridana serum inhibited liquefaction of gelatin by the gram-negative bacterium, Pseudomonas aeruginosa.

We have demonstrated, among members of the genus Lampropeltis, variation of serum-mediated venom-neutralizing properties. Although we found no apparent relationship between variation of these properties and sex, sibling status, or age of the specimens studied, the possibility of individual variation within a population cannot be discounted. Dessauer and Pough (1975) reported variation of a number of blood proteins (e.g., albumin, hemoglobin) among eastern and western populations of six taxa of L. getulus. Venom neutralizing proteins are probably subject to similar variability. A study such as ours, using larger sample sizes, could establish the nature and frequency of variability.

Serum from nominal L. getulus and L. g. floridana had the widest effective spectrum of neutralization in mice. Interestingly, Blaney (1977) considered L. g. getulus as derived from L. g. floridana. Venom neutralizing potential of serum from L. m. greeri and L. alterna showed few similarities, but several venoms (C. v. viridis, S. m. barbouri, A. c. mokasen, and A. p. conanti) were similarly neutralized by serum from L. t. triangulum and L. ruthveni. Lampropeltis alterna has been regarded as a full species (Brown, 1901; Blanchard, 1921; Smith and Taylor, 1945; Garstka, 1982) or as a race of L. mexicana (Gehlbach and Baker, 1962; Gehlbach and McCoy, 1965; Tanzer, 1970). Lampropeltis m. greeri has presented similar taxonomic confusion (Gehlbach and Baker, 1962; Garstka, 1982). Lampropeltis ruthveni (Blanchard, 1921; Smith and Taylor, 1945; Garstka, 1982) is a problematical species that has been considered synonymous with L. t. arcifera (Williams, 1988).

None of the serum samples tested showed any capacity to neutralize M. f. fulvius venom. Most micrurine venoms (including M. f. fulvius) contain myolytic phospholipases A₂ and postsynaptic polypeptide neurotoxins. Venom from type A C. s. scutulatus contains high concentrations of Mojave toxin. Of the samples tested, serum from only four species (L. g. getulus, L. g. floridana, L. calligaster, and L. t. triangulum) demonstrated any neutralization potential for type A C. s. scutulatus venom, while all could neutralize type B venom, which was hemorrhagic and lacked Mojave toxin. None of these species is sympatric with C. s. scutulatus type A populations. Although the data suggest that the aforementioned species can neutralize a natural envenomation inflicted by a type A C. s. scutulatus, observations reported previously suggest that injection of other venoms containing high concentrations of neurotoxins resulted in rapid death (Ditmars, 1910, 1937). The neural systems of squamates that have been investigated (primarily scincid lizards) function analogously to those of mammals (White, 1976; Wood and Lenfant, 1976), and therefore, are probably similarly susceptible to neurotoxins. It is noteworthy, however, that some workers have found that neuromuscular transmission of some ophidian genera are not affected by high doses of postsynaptic neurotoxins (Burden et al., 1975; Liu and Xu, 1990). Thus, some ophidian species may have marked resistance to some neurotoxins due to characteristics (e.g., toxin-resistant cholinergic receptors) inherent in neuromuscular transmission. In these species, serum immunity against neurotoxins may be unimportant or may provide an additional protective mechanism. It remains to be determined if Lampropeltis species exhibit such characteristics of the peripheral neural system.

Low neutralization potential was found when most serum samples were assayed with C. v. Kelleri venom. This venom contains hypotensive peptides, which cause transient increases in vascular permeability and precipitous hypertension leading to shock (Schaeffer et al., 1978; Russell, 1980). It is possible that venom-neutralizing components of Lampropeltis serum have a low efficacy for these toxins or their target sites. It remains to be determined if injection of Lam-
propeltis with these toxins would cause vascular subdivision of sarcoplasmic reticulum and the perinuclear space, producing vacuolation and sarcoplasmic degeneration (Owensby et al., 1976; Cameron and Tu, 1978; Owby, 1982). It is unclear if serum from any Lampropeltis could specifically neutralize this myotoxic fraction. All of the serum samples studied had effective neutralization potential for the C. v. viridis venom used in the assays. It remains undetermined whether this commercial pooled sample contained high concentrations of myotoxic a. All mice injected with mixtures of any serum tested and appropriate doses of M. f. fulvius venom, exhibited myoglobinuria indistinguishable from those injected with venom alone. This suggests that elapid venom myolytic phospholipases A, are not effectively neutralized by Lampropeltis serum. The effect of such myolytic fractions upon ophidian musculoskeletal elements is unstudied.

Marked variation was observed also in neutralization potentials of Lampropeltis serum for the venoms with potent hemorrhagic activities. Most Crotalus venoms studied to date produce rhexic hemorrhage (Owby, 1982), which is characterized by destruction of the vascular wall. Hemorrhage per diapedesis is characterized by modification of intercellular junctions, allowing blood components to escape without alteration of endothelial cell morphology (Owby, 1982). Agkistrodon c. mokasen venom causes edema, limited hemorrhage, and lymphocyte infiltration (Wingert et al., 1980). Sistrurus m. barbouri venom is hemorrhagic (Friederich and Tu, 1971), but there are no data describing identity of the hemorrhagins present in this venom. Variation of Lampropeltis serum neutralization potential for different hemorrhagic venoms is probably due to the nature of hemorrhagin action (rhexic vs. diapedetic), the efficacy of hemorrhagin neutralizing serum proteins for a specific venom hemorrhagin, and/or the amount of the antihemorrhagin fraction present in serum of a given species.

The mechanism of protection against most of the crotaline venoms assayed appears to be a neutralization of hemorrhagic and proteolytic activities and/or a blockage of receptor sites for these venom components on target tissues. Philpot et al. (1978) isolated an antiproteolytic fraction from L. getulus ssp. serum that inhibited bradykinin release from human serum, an activity these investigators associated with lethality. Other workers have proposed antibody neutralization of venom proteases as the mechanism of protection (Vellard, 1950; Bonnett and Gutman, 1971). A humoral mechanism of immunity seems unlikely considering that many of the antiproteolytic-antihemorrhagic fractions that have been isolated from serum of venomous and non-venomous ophidians, behave as a-globulins or albumin (Clark and Voris, 1969; Omori-Satoh et al., 1972; Ovadia, 1978; Tomihara et al., 1988; Weinstein et al., 1991; Weissenberg et al., 1991). We found that E. g. guttata serum had neutralization potential for several crotaline venoms. Philpot (1954) reported that E. quadrivirgata serum protected mice against venoms of Trimersurus flavoviridis and A. contortrix. Although we found that serum from P. m. sayi and an allied species, R. i. antonii, lacked significant neutralization potential for the venoms tested, Harvey (1960) described inhibition of C. atrox venom proteases by P. m. sayi serum. Biochemical and morphological data have indicated that Lampropeltis is allied with Elaphe, Pitophis, Cemophora, and Arizona (Underwood, 1967; Williams and Wilson, 1967; Blaney, 1973, 1977; Dowling et al., 1983). Dowling et al. (1983) recognized the colubrine group of these five genera as the tribe Lampropeltini. Serum proteins of these genera often produce patterns indistinguishable in crossed immunoelectrophoresis (Minton and Salanitro, 1972), and albumin relationships are very close (Dowing et al., 1983), suggesting little immunological distance exists between these taxa. Lampropeltis getulus, L. calligaster, L. alterna, and numerous Elaphe sp. all have a karyotype of 2N = 36 (Bury et al., 1970; Baker et al., 1972). Vertebral morphology is also similar between the two genera (Garstka, 1982). On the basis of numerous morphological characters, Underwood (1967) considered Lampropeltis as a form derived from Elaphe.

The results of our study with Elaphe and Rhinocelotus sera, as well as the data of Philpot (1954) regarding E. quadrivirgata serum immunity, suggest that these genera may all share some degree of serum immunity against certain venoms. Venom-neutralizing serum proteins may be a trait conserved in the manner of some of the other biochemical and morphological characteristics outlined above.

Although many of the Lampropeltis sp. whose sera were investigated here, are sympatric with some of the venomous species whose venoms were included in this study, little relationship seems apparent between status of neutralization potential and feeding ecology. Prey items reported for different Lampropeltis have included small mammals, birds and their eggs, lizards,
snakes and their eggs, and amphibians (Blanchard, 1921; Mecham and Milstead, 1949; Smith, 1950; Axtell, 1951; Lockwood, 1954; Wright and Wright, 1957; Klimstra, 1959; Fitch and Fleet, 1970; Tanzer, 1970; Minton, 1972; Conant, 1975; Williams, 1988). Most ophiophagous prey recovered from stomach contents have been colubrines, xenodontines, or natricines (Klimstra, 1959; Fitch and Fleet, 1970). Lockwood (1954) reported that specimens of L. c. rhombomaculata accepted Carphophis, Storeria, and Opheodrys, but refused A. contorta. Minton (1972) reported a specimen of L. g. niger from Indiana with an A. c. mokasen in its stomach. Pope (1944) proposed that mammals were preferred prey of L. trian
gulum, with snakes accepted as an alternate.

Although the dietary profiles of many Lampropeltis appear to lack venomous snakes as important prey items, it is probable that feeding ecology of a given isolated population is most important in the genetic expression of venom-neutralizing serum proteins. The neutralizing proteins may be widespread among different Lampropeltis populations, due to conservation of a gene that evolved in a Lampropeltis ancestor during a period of heightened predation upon venomous ophidians. These proteins may be expressed in high concentrations among specific, isolated populations that still prey regularly upon venomous snakes.

Further study is warranted of venom neutralizing properties among colubrid genera. Other ophiodagous genera such as Drymarchon, Masticophis, etc. could be examined for venom neutralizing serum components and their relationships to those of Lampropeltis and Elaphe. Such investigations could characterize the proteins responsible for serum immunity, and determine their biochemical relationships among these genera.

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The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense (para. 4-3, AR 360-5).

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The Effect of Water Salinity on Growth and Oxygen Consumption of Snapping Turtle (Chelydra serpentina) Hatchlings from an Estuarine Habitat

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ABSTRACT.—Snapping turtle eggs were collected from contiguous brackish and lower brackish/fresh water habitats within a Long Island, New York estuary. Eggs were also collected from a remote inland fresh water site. Hatching snapping turtles were exposed to fresh water, 10, 20, 25 and 40% seawater (100% seawater = 35 ppt) for periods varying from 5 to 20 weeks. There were no significant differences in terms of salinity in growth optima between hatching turtles obtained from contiguous habitats of differing osmotic stress. The estuarine group of turtles as a whole grew significantly better in brackish water (up to 25% seawater) as compared to fresh water. Comparison with hatchlings obtained from an inland fresh water site suggests that observed differences in growth in the estuarine group are due primarily to a marked inability to grow in fresh water rather than enhanced growth in saline water. Salinity did not affect the rate of oxygen consumption of the turtles.

Coastal reptiles appear to be under selective pressure for the development of physiological adaptations for the estuarine environment (Dunson and Mazzotti, 1989). Dunson (1986) showed that snapping turtle (Chelydra serpentina) hatchlings from coastal Virginia grew faster in brackish water than in fresh water, while inland (New Jersey) hatchlings grew faster in


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