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

SCOTT A. WEINSTEIN, CLEMENT F. DEWITT, AND LEONARD A. SMITH

Department of Immunology and Molecular Biology, Toxinology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011, USA

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 ophiophagic and non-ophiophagic, aglyphic, or opisthomegadont 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 ophiophagous 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 *Lampropeltis* with a representative selection 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. alterna* (one specimen, male), *L. mexicana greeri* (two specimens, one male, one female), *L. ruthveni* (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, and 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.) LD_{50} 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 LD_{50} was calculated by the Spearman-Karber method (World Health Organization, 1981). The fiducial (95% confidence) limits for the LD_{50} were determined.

Estimation of Protein Content.—Protein content was determined using the bicinchoninic acid assay (BCA assay, Pierce Chemicals, Rockford, Illinois; Smith et al., 1985).

Venom Neutralization Assays.—Venom solutions that contained 4.0 to 21.0 LD_{50} 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 venom mixed 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 LD_{50} 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 LD_{50} 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. LD_{50} 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.

Species (locality)	i.p. lethality (murine LD_{50}, mg/kg) [95% confidence limits]	LD_{50} values reported previously (i.p. murine LD_{50}, mg/kg)
Crotalus adamanteus (Tampa, Florida)	3.30 [2.92–3.74]	1.89,[1] 3.75,[2] 2.7[3]
C. *atrox* (Archer Co., Texas)	4.79 [4.07–5.64]	6.0,[4] 8.42,[5] 4.5[3]
C. *viridis viridis* (Texas)	2.37 [2.09–2.70]	2.25,[4] 2.0,[7] 2.25[3]
C. *v. helleri* (California)	1.84 [1.53–2.20]	1.56,[5] 2.44,[7] 2.8[3]
C. *scutulatus scutulatus* (type A, Yuma, Arizona)	0.17 [0.143–0.199]	0.18,[7] 0.23,[8] 0.13–0.54[3,9]
C. *s. scutulatus* (type B, unknown)	2.72 [2.29–3.23]	2.3–3.8$_A$[3]
C. *horridus horridus* (unknown)	3.31 [2.89–3.80]	2.91,[8] 2.69[10]
Sistrurus m. barbouri (Florida)	9.40 [8.59–10.29]	6.0,[4] 6.84[7]
Agkistrodon contortrix mokasen (Frederick Co., Maryland)	15.06 [13.57–16.71]	7.8,[11] 8.9,[3] 10.50[8]
A. piscivorus conanti (Tampa, Florida)	5.33 [4.88–5.82]	5.10,[8] 5.11$_B$[11]
Micrurus f. fulvius (Florida)	0.62 [0.53–0.73]	0.67,[8] 0.9[12]

[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. s. 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 LD_{50} 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. holbrooki, 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. 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 LD_{50} 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

TABLE 2. Neutralization potential of *Lampropeltis* serum. Data represent the potential number of venom LD_{50} neutralized in mice/ml serum. Calculated from 750 μg serum protein from each species mixed with appropriate venom doses. See Materials and Methods for details of assays. *Lampropeltis t. triangulum* was assayed with 1 mg of serum protein. ND = not determined.

Serum source	Serum protein content (mg/ml serum)	Venom source (locality)											
		Crotalus atrox (Archer Co., Texas)	*C. adamanteus* (Tampa, Florida)	*C. viridis viridis* (Texas)	*C. v. helleri* (California)	*C. scutulatus scutulatus* (type A) (Yuma, Arizona)	*C. s. scutulatus* (type B) (unknown)	*Crotalus horridus horridus* (unknown)	*Sistrurus miliarius barbouri* (Florida)	*Agkistrodon contortrix mokasen* (Maryland)	*A. piscivorus conanti* (Florida)	*Micrurus fulvius fulvius* (Florida)
Lampropeltis getulus getulus	93	279	279	310	124	124	496	248	124	124	155	0
L. g. californiae (striped phase)	78.5	183	183	157	0	0	471	262	209	235	183	0
L. g. californiae (banded phase)	54	108	108	180	90	0	72	208	108	162	162	0
L. g. holbrooki	74	148	197	222	74	0	173	247	98	247	345	0
L. g. splendida	52	104	69	104	69	0	243	243	104	156	0	0
L. g. floridana	89	207	267	237	119	119	376	282	118	267	345	0
L. triangulum triangulum	64.5	213	129	161	ND	64	ND	181	97	161	177	0
L. t. hondurensis	47	63	156	94	0	0	219	125	63	172	141	0
L. calligaster	70	140	233	163	93	93	327	233	93	140	186	0
L. mexicana greeri	62.5	125	145	125	83	0	187	205	83	187	125	0
L. alterna	87	116	0	174	0	0	319	116	174	232	203	0
L. ruthveni	78	104	104	156	104	0	286	156	104	156	182	0

no potential to neutralize venom from the congeneric, *A. p. conanti* (Table 2).

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. horridus*, and *A. c. mokasen* (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. californiae* (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 LD_{50} *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 LD_{50} 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 LD_{50}/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, and 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 prostration 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. californiae*, 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

doses within 3 weeks. Other reports indicate, however, that some individual specimens are susceptible to lethal effects of some venoms. Allyn (1937) described how a *L. g. getulus* expired within 1 h after being envenomated by a *S. catenatus*. Keegan and Andrews (1942) killed a young adult *L. calligaster* with a large dose (767 mg/kg) of *A. contortrix* venom. Death occurred 52 days after administration of the venom bolus. Our data indicate that serum from *L. g. getulus* and *L. calligaster* had relatively low neutralization potential for venoms of *S. m. barbouri* and *A. c. mokasen*, respectively. Venom of *S. catenatus* has a much higher lethal index (0.22 mg/kg, i.p., Minton, 1956) than *S. m. barbouri* venom (6.0–9.4 mg/kg, i.p., Table 1).

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 A_2 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. helleri* venom. This venom contains hypotensive peptides, which cause transient increases in vascular permeability and precipitous hypotension 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 vasculotoxic effects. Klauber (1956) reported immunity of *L. g. californiae* to *C. v. helleri* natural envenomation.

Venom of *C. v. viridis* contains myotoxin a, a non-enzymatic polypeptide which causes dilation of sarcoplasmic reticulum and the perinuclear space, producing vacuolation and sarcoplasmic degeneration (Ownby et al., 1976; Cameron and Tu, 1978; Ownby, 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 myotoxin 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_2 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 (Ownby, 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 (Ownby, 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 Guttman, 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 α-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 *Trimeresurus flavoviridis* and *A. contortrix*. Although we found that serum from *P. m. sayi* and an allied species, *R. l. 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*, *Pituophis*, *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 (Dowling 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 *Rhinocheilus* 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 ophidian 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. contortrix.* 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. triangulum,* 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 ophiophagus 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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