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MOJAVE RATTLESNAKE *CROTALUS SCUTULATUS SCUTULATUS* VENOM: VARIATION IN TOXICITY WITH GEOGRAPHICAL ORIGIN*

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IT IS generally recognized that the LD_{50} values of Mojave rattlesnake (*Crotalus scutulatus scutulatus*) venoms are lower than other North American rattlesnake species (GITHENS and GEORGE, 1931; MINTON, 1959; *Poisonous Snakes of the World,* 1968); although *Crotalus viridis concolor* produces venom approximating the LD_{50} of *C. s. scutulatus* (GLENN and STRAIGHT, 1977). *Crotalus s. scutulatus* was described first by KENNICOTT (1861) and according to KLAUBER (1931; 1971; 1972) there was a long standing confusion among collectors and taxonomists in distinguishing *scutulatus* from *atrox* because of their similarity in appearance and overlap in distribution. These two venoms are distinctively different in lethality as determined by laboratory mouse LD_{50} values (*C. atrox*—3·71 mg/kg vs *C. s. scutulatus*—0·23 mg/kg; GITHENS and GEORGE, 1931; *Poisonous Snakes of the World,* 1968). Also, the clinical symptoms of *scutulatus* envenomation are quite distinct from *atrox* envenomation. Sometimes *C. s. scutulatus* envenomation produces local swelling, edema and pain whereas other cases show very few local symptoms and signs but severe systemic changes (RUSSELL, 1969; RUSSELL and PUFFER, 1970; RUSSELL *et al.,* 1975). The major lethal activity of *Crotalus s. scutulatus* venom has been reported to be an acidic cardiotoxic protein (BEIBER *et al.,* 1975; TU *et al.,* 1976) and conversely a basic neurotoxic protein (PATTABHIRAMEN and RUSSELL, 1975; HENDON, 1975). The studies reported here indicate that within the subspecies, *Crotalus s. scutulatus*, venom from specimens collected in southern California and southwestern Arizona is distinctly different in lethality and reactivity with the commercial antivenin from venom of specimens collected in central and northwest Arizona. This geographical variation within the subspecies could explain some of the confusion about the clinical aspects of *scutulatus* bites.

Specimens of *Crotalus scutulatus scutulatus* (KENNICOTT), from southern California and southwestern Arizona were supplied by a commercial supplier (Western Zoological Supply, Jim Brockett, Monrovia, California) along with collection data. Specimens from central and northwest Arizona were acquired or collected by our laboratory personnel. Venoms were collected from individual specimens by manual venom extraction techniques and centrifuged at 1000 *g* for 15 min. The supernatent fluid was frozen, lyophilized to dryness and stored at —4°C. All toxicity and neutralization capacity tests were compared on a protein weight basis. Total protein was determined by the biuret method of GORNALL *et al.* (1949) for all venom, plasma and antivenin samples. The LD_{50} assays of individual venom samples were performed by injecting graduated doses intraperitoneally (i.p.) into 18–22 g white,

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Swiss–Webster mice. LD_{50} data was obtained from not less than 5 mice per dose and 4 doses per test. The LD_{50} results were calculated by the moving-average interpolation of KARBER (1931) and THOMPSON (1947). Neutralization capacity tests were performed by mixing a constant amount of protein of commercial antivenin (Wyeth Antivenin Crotalidae polyvalent) or *Crotalus atrox* heated plasma (STRAIGHT and GLENN, 1976), with graduated venom doses and the mixture injected i.p. within 30 min. The untreated venom i.p. LD_{50} values were compared to the i.p. LD_{50} values of the antivenin or plasma treated venom, in mice and the greater the LD_{50} value of the treated venom the greater the neutralization. *Crotalus atrox* blood was collected by cardiac puncture or decapitation into sodium citrated (3·8%, 4 parts blood to 1 part sodium citrate) tubes. The blood was centrifuged at 1000 *g* for 15 min and the plasma was collected. The *Crotalus atrox* plasma was then heated in a water bath at 56–58°C for 30 min to remove its toxicity and stored frozen at —40°C until used.

A total of 38 specimens of *Crotalus scutulatus scutulatus* were tested individually for venom LD_{50} values, 19 from southern California, 17 from the north central and northwestern half of Arizona, and 2 from southwestern Utah. One specimen of *Crotalus scutulatus salvini* was available for testing and represents the only report on mouse LD_{50} data of this subspecies to the best of our knowledge (Table 1). All California *C. s. scutulatus* specimens produced venom i.p. LD_{50} values within the range of 0·13–0·33 mg/kg mouse for 18–22 g mice (Venom A—Table 1). Nine of the 17 Arizona specimens produced venom i.p. LD_{50} values within the range of 0·13–0·54 mg/kg mouse (Venom A—Table 1). The mean of these two groups combined was 0·24 mg/kg. The LD_{50} values of *C. s. scutulatus* venom-A are consistent with the reports of MINTON (1959) and RUSSELL (1962). However, 8 *C. s. scutulatus* specimens from the northeastern extreme of their range in Arizona exhibited venom of considerable higher LD_{50} values, 2·3–3·5 mg/kg mouse (venom B—Table 1). Experiments involving repeated venom extractions of the venom B, *C. s. scutulatus* specimens, consistently reproduced the same LD_{50} values.

TABLE 1. COMPARISON OF INTRAPERITONEAL LD_{50} VALUES (18–22 g MICE) OF *Crotalus scutulatus* VENOM COLLECTED FROM INDIVIDUAL SPECIMENS OF VARIABLE GEOGRAPHICAL ORIGIN

Venom*	No. of specimens	I.P. LD_{50} (range) (mg/kg)
C.s. scutulatus–A (Calif.-Arizona)	28	0·24 (0·13–0·54)
C.s. scutulatus–B (Arizona)	8	2·80 (2·3–3·8)
C.s. scutulatus–A (Utah)	2	0·11 (0·09–0·12)
C.s. salvini	1	0·18

*Venom-A from specimens from southern California and south-west Arizona. Venom-B from specimens from northwest and north central Arizona.

Additional evidence of the distinctive nature of the two venoms was obtained by testing the efficacy of the commercial antivenom and *Crotalus atrox* plasma in neutralizing each venom (Table 2). *Crotalus atrox* plasma, known to markedly increase the LD_{50} values of several crotalid venoms (STRAIGHT and GLENN, 1976), and commercial antivenin (Antivenin Crotalidae Polyvalent) markedly increases the LD_{50} of the venom-B. However, the venom-A samples were by comparison poorly neutralized by both products (Table 2; also see STRAIGHT and GLENN, 1976).

TABLE 2. LD_{50} VALUES OF CROTALID VENOMS ALONE AND VENOMS NEUTRALIZED BY WYETH ANTIVENIN AND *Crotalus atrox* PLASMA IN 18–22 g WHITE SWISS WEBSTER MICE

Species	LD_{50} VALUES (mg/kg) Venom alone	Wyeth and venom	CAAP and venom
C. s. scutulatus (Venom "A")	0·15	1·75	1·5
C. viridis concolor	0·20	2·6	2·8
C. d. terrificus	0·20	4·15	2·95
C. adamanteus	2·7	8·15	13·25
C. atrox	4·5	23·8	21·95
C. s. scutulatus (Venom "B")	3·5	40·0	40·0

All tests were done on a protein (biuret) weight basis on a single venom sample comparing venom alone, venom + 10 mg Wyeth antivenin and venom + 10 mg *C. atrox* (heated) plasma (CAAP).

The differences in venom yield of males and females (Table 3) seems to be related to the length of the snakes. The males attain greater length, hence larger head and venom gland size. KLAUBER (1972) reports the maximum venom yield (dry weight) of *Crotalus s. scutulatus* to be 141 mg and the average yield per fresh adult to be 77 mg, using combined sexes.

Geographical variation in reptile morphology and behavior at the *subspecies* level is well known, but little has been reported concerning subspecific geographical variation of venoms. *Crotalus viridis concolor* venom is considerably more toxic than the other subspecies in the *viridis* group (GLENN and STRAIGHT, 1977) indicating that tri-nomenclature (sub-species) should be used whenever possible in all toxicological studies. Also, the geographic origin of the specimens from which the venom was obtained should be reported. Additional taxonomic study may reveal more definitive information on distinctive characteristics of venom-A specimens vs venom-B specimens, other than their venoms. The facts are as follows: (1) The venom-B specimens, are definitely members of the *Crotalus s. scutulatus* variety as we presently consider the sub-species. (2) All eight venom-B specimens exhibited coloration in the form of darker brown background and dorsal markings, whereas the venom-A specimens exhibited a lighter yellowish–greenish background coloration. Understandably coloration is a very poor criteria for identification and could be very misleading. KLAUBER (1972) reported that "tan or brown" specimens did occur in Arizona. (3) *Crotalus s. scutulatus* specimens producing the venom-B samples were all from the extreme northeastern portion of this rattlesnake's distribution (Phoenix area).

TABLE 3. CHARACTERISTICS OF *Crotalus scutulatus scutulatus* VENOM FROM INDIVIDUAL SPECIMENS* (16 MALES AND 13 FEMALES)

Characteristic	Sex	Range	Mean
Total length (cm)	Males	60·5–110·0	85·5
	Females	52·0–87·5	67·5
Venom dry weight (mg)	Males	15–139	61·6
	Females	8–45	22·9
Protein (% dry wt)	Males	93–98·5	96·0
	Females	89–98	94·5

*Specimens of both sexes under 50 cm were not included.

This raises two questions. First, if a distinctive geographical distribution exists, what percentage of the population produce venom-B, and second, are there enough taxonomic differences for sub-species ranking? Only two sub-species of *C. scutulatus* are recognized at this time, *C. s. scutulatus* and *C. s. salvini*. The phylogenic and classification implications of these findings may be important for those involved in the medical management of snakebite and in venom research.

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