Stress and the Toxicity of Venoms

Abstract. *Animals injected with venom of the scorpion* Centruroides sculpturatus *Ewing or venom of the rattlesnake* Crotalus atrox *Baird and Girard were subjected to high- and low-temperature stress. Unconditioned animals transferred to a modified temperature were less refractory to the venoms than those conditioned for 48 hours, but all animals stressed were less refractory than unstressed animals. Animals receiving a series of small doses of epinephrine were similarly affected. This apparent change in toxicity of the venoms seems to be due to the physiological effects of stress rather than to the temperature per se.*

The toxicity of a venom may vary with the ambient temperature of the test animal (*1, 2*). Whether this variance is due to the ambient temperature or the concomitant stress is not clear (*1*). Our experiments were designed to determine whether stress induced by changes in temperature would affect the 50-percent lethal dose (LD_{50}) of two venoms.

Scorpions, *Centruroides sculpturatus* Ewing, and rattlesnakes, *Crotalus atrox* Baird and Girard, were milked in the laboratory, and the venom was subjected to lyophilization and then reconstituted with de-ionized, distilled water at the following concentrations: scorpion venom, 5 mg/ml and rattlesnake venom, 10 mg/ml.

Holtzman/Sprague-Dawley strain of rats of both sexes weighing between 100 to 150 g and free of rales were used in the bioassays. Food was withheld for 24 hours before assay, water for 2 hours. The rats subjected to temperatures of 35° to 38°C had water before them continuously. All animals were given water 2 hours after injection of venom, and those that survived 12 hours were offered food. The animals were observed over a period of 24 hours.

The scorpion venom was injected subcutaneously and the rattlesnake venom intraperitoneally. The LD_{50} of the scorpion venom for rats was 1.00 mg/kg with a confidence interval of 0.88 to 1.14 mg/kg; that of the rattlesnake venom was 15.0 (10.0 to 22.0) mg/kg.

The statistical design of this study followed that suggested by Thompson and Weil (*3*) and Wadsworth (*4*) with $K = 3$, $n = 4$, and $R = 2$. All assays were replicated until adequate verification was evident.

The rats were divided into three groups of 48, plus a control group of 16. One group was subjected to a temperature of 2°C, another to 13°C (both at 30 to 40 percent relative humidity), a third group to 35° to 38°C (25 to 35 percent relative humidity) and the control group to 24° to 27°C (25 to 35 percent relative humidity). Each of the experimental groups was further divided as follows:

1) Those kept at the modified temperature for 48 hours before administration of the venom. These were further subdivided into group 1A, consisting of those injected and observed for 24 hours at the same temperature, and group 1B, consisting of those injected but 10 minutes later removed to normal temperature.

2) Those kept at normal temperature before venom was administered and removed 10 minutes later to the modified temperature.

Since epinephrine is released under stress conditions (*5*), additional verification for the action of stress was determined in the following manner:

1) One group of 16 rats for each venom was injected subcutaneously with 2.0 mg/kg (concentration 1 mg/ml) in one dose 20 minutes after the venom was injected. The LD_{50} of this epinephrine was 7.07 (4.3 to 11.5) mg/kg by the same route.

2) Another group of 16 rats was injected subcutaneously with ½ LD_{50} (3.54 mg/kg) of epinephrine in one dose 20 minutes after the venom injection.

3) In order to maintain the epinephrine concentration in the rat body during the 1st hour of assay, another group of 16 rats received the same quantity (½ LD_{50}) of epinephrine in four equal doses subcutaneously, the first dose 20 minutes after the venom injection and the other three at 10-minute intervals.

Table 1 gives the results for venom toxicity obtained when rats injected with venom were subjected to different temperatures. Toxicity is measured by the LD_{50} and delimited by a 95-percent confidence interval (CI).

Table 2 gives the effect of epinephrine on venom toxicity when the former is given as a single dose or in multiple doses.

In the reciprocal test for synergism between epinephrine and the venoms, ½ LD_{50} of the venom in each case gave a significant increase in the toxicity of epinephrine. The scorpion venom changed the epinephrine LD_{50} from 7.07 mg/kg (CI, 4.3 to 11.5) to 2.98 mg/kg (2.10 to 4.20) and the rattlesnake venom changed it to 1.49 mg/kg (0.88 to 2.53).

Both venoms seemed to be more toxic in rats at either high or low temperature. Selye (*5*) states: "It would be wrong to attribute specifically to cold a change produced by low temperature if heat exerts the same effect." Therefore, temperature, as Carmichael (*1*) indicated, does not seem to be the direct cause for modification of toxicity.

The data indicate that the changes in toxicity of scorpion venom were due to stress. Mere exposure to a modified ambient temperature did not give a statistically significant increase

Table 1. Venom toxicity in relation to ambient temperature.

Group	Venom*	LD_{50} and 95-percent confidence interval (mg/kg)			
		2°C	13°C	24° to 27°C	35° to 38°C
		Group 1, 48 hours of conditioning			
1A. Retained at same temp. after injection	R	6 (5–9)	9 (6–13)	15 (10–22)	8 (6–12)
	S	0.56 (0.50–0.64)	0.76 (0.64–0.88)	1.00 (0.88–1.14)	0.60 (0.52–0.68)
1B. Removed to normal temp. after injection	R	18 (13–25)	13 (9–18)	15 (10–22)	13 (9–18)
	S	1.59 (1.34–1.90)	1.27 (1.13–1.43)	1.00 (0.88–1.14)	0.90 (0.80–1.00)
		Group 2, kept at normal temperature			
2. Removed to modified temp. after injection	R	3 (2–4)	6 (5–9)	15 (10–22)	8 (6–12)
	S	0.56 (0.50–0.64)	0.63 (0.55–0.72)	1.00 (0.88–1.14)	0.60 (0.52–0.68)

* R, rattlesnake, *Crotalus atrox*; S, scorpion, *Centruroides sculpturatus*.

in toxicity. The rats had to remain at the modified ambient temperature (under the influence of the stressor) after the venom was injected. For example, whether they were in the low ambient temperature previous to injection (group 1A) or whether they were placed in the low ambient temperature after injection (group 2) did not seem to be important. But those animals that were removed from the stressor after injection (group 1B) did not experience a significant increase in toxicity; in fact, some appeared to be more refractory to the venom (especially at 2°C). "Exposure to cold is followed by an increased production of certain hormones such as corticotropin, corticoids, thyroid hormone and epinephrine" (*5*). Since epinephrine is rapidly inactivated in the body (*6*) the reaction of those animals removed from the influence of the stressor would certainly suggest that this hormone could be involved in the increase in toxicity. This reasoning is supported by the results obtained when epinephrine was injected in varying doses after venenation of the test animals. This apparent synergism of epinephrine with both venoms is further substantiated by the reciprocal test in which the LD_{50} of epinephrine was reduced from the normal 7.07 mg/kg (4.3 to 11.5) to 2.98 mg/kg (2.1 to 4.2) by scorpion venom and to 1.49 mg/kg (0.88 to 2.53) by rattlesnake venom.

The increase in refractoriness to scorpion venom and rattlesnake venom by those animals in group 1B (Table 1) further supports the role of stress, especially at the 2°C. With the destruction of the epinephrine, the synergistic action of this hormone and the venom is discontinued. Now the organism's systemic defense (counter shock) comes into play, and a resistance to the venom action seems to occur. The action of this mechanism is not clearly understood (*7*), but the therapeutic implications are interesting. At the other temperatures (13°C and 35° to 38°C) the initial stress was apparently not as great and the resulting counter shock was of lower degree. At 35° to 38°C for scorpion venom and at both 13°C and 35° to 38°C for rattlesnake venom, it is possible that sufficient residual epinephrine prevented the LD_{50} from going above normal levels.

Although some of the results with rattlesnake venom (Table 2) do not show statistical significance, the decrease in the LD_{50}'s with the increase in stimulus is sufficient to establish significance. Also, the results obtained with epinephrine add to the weight of this conclusion.

Changes in venom toxicity seem to occur when the recipient rats are subjected to changes in ambient temperature. Whether the change is an increase or a decrease in temperature does not seem to be important; the greater the temperature change the greater the change in toxicity. Epinephrine also increased the toxicity of these two venoms in rats. Since this hormone is released under conditions of stress, the mechanism causing the increase in toxicity of these venoms during stress seems to be, at least in part, a result of the synergism between the venoms and epinephrine.

"It is now definitely established that nervous stressors (pain, emotions) are particularly conducive to the development of the somatic manifestations of the stress syndrome, so that stress can be both cause and be caused by mental reactions" (*8*) and epinephrine is a concomitant product of stress regardless of the source. This evidence, plus the evidence provided by the present research, should call for a reevaluation of certain recommended therapeutic practices for the treatment of snake venenation. Thus, practices increasing the pain and the complete immobilizing of the patient would not provide the optimum therapeutic environment. In fact, pain and emotional stressors could be the cause of a continuous production of epinephrine.

Table 2. Effect of epinephrine on venom toxicity.

Injection	LD_{50} and 95-percent confidence interval (mg/kg)	
	Scorpion	Rattlesnake
Venom only	1.00 (0.88–1.14)	15 (10–22)
Venom plus 2.0 mg/kg epinephrine in one dose 20 min after venom	0.94 (0.79–1.12)	14 (10–20)
Venom plus ½ LD_{50} epinephrine (3.54 mg/kg) in one dose 20 min after venom	0.67 (0.56–0.79)	9 (8–11)
Venom plus ½ LD_{50} epinephrine (3.54 mg/kg) in four equal doses, the first 20 min after venom, other three at 10-min intervals	0.44 (0.36–0.55)	6 (5–7)

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Dendroctonus pseudotsugae: A Hypothesis Regarding Its Primary Attractant

Abstract. *The Douglas-fir beetle is attracted to α-pinene but repelled by β-pinene. Attacks on standing trees are related to the content of α- or β-pinene in the tree; this correlation suggests that volatile oils may be the stimuli initially attracting the insect to the Douglas fir.*

The Douglas-fir beetle (*Dendroctonus pseudotsugae* Hopkins) exhibits positive and negative motor responses to selected volatile oils of Douglas fir [*Pseudotsuga menziezii* (Mirb.) Franco], and these motor responses vary with the composition and concentration of the volatile oils. Perhaps it is of greater importance that in standing trees the composition and concentration of the volatile oils attractive to the insect can be related to the parts of the trees attacked.

The attractiveness of selected volatile oils was tested by placing five beetles and a given oil in a closed-system olfactometer (*1*). The position of the beetles in relation to the oil was recorded every 5 minutes for 1