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# ROLE OF METALS IN SNAKE VENOMS FOR HEMORRHAGIC, ESTERASE AND PROTEOLYTIC ACTIVITIES*

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**Abstract**—Atomic absorption analysis was used in the determination of metal distribution of seventeen snake venoms. Ca, Mg, Zn, Na and K, were found in all venoms tested. Biological and enzymatic activities were followed as the metal ions of the venoms were removed with EDTA. Lethality and esterase activity remained unchanged by the removal of metals, while hemorrhagic and proteolytic activity showed a marked decrease. Hemorrhagic activity could be partly restored by the addition of divalent Mg or Zn.

LOCAL tissue damage such as hemorrhage and necrosis is a typical symptom due to Crotalidae and Viperdae envenomation. It has been reported that some chelating agents reduce the hemorrhagic and lethal effects of snake venoms.[1–3] Goucher and Flower[4] detected loss of hemorrhage and local necrosis upon addition of EDTA to the venom of eastern cottonmouth, *Agkistrodon piscivorus piscivorus*. The findings of these researchers strongly suggests that certain metals are involved in venom hemorrhagic activity. At present, snakebite treatment depends exclusively on the injection of horse serum antivenin. In many cases, local tissue damage is not prevented by the use of antivenin unless it is administered immediately. Therefore, the chelating agents which inactive hemorrhagic action of snake venoms, may be useful for chemical treatment.

Devi[5] reported that iron content of Indian cobra (*Naja naja*) and Russell's viper (*Vipera Russellii*) venoms was 0·028 and 0·016 mg% respectively. Zinc was also found in Indian Cobra venom. Moav *et al.*[6] reported that copper and zinc were found in high concentration in *Vipera Palestina* venom after simple dialysis. However, no systematic analysis of metal content in snake venom or its relation to biological activity has been reported.

In order to clarify the role of metals in venom hemorrhagic activity we have analyzed for the metals contained in various snake venoms. After the preliminary analysis, the venoms were treated with EDTA, and the metals of these venoms were again determined. Lethality, hemorrhage and several enzyme activities of the venoms were tested before and after EDTA treatment. To further establish the role of metal ion, Ca, Mg or Zn was added to the EDTA-treated venom and its effect on the hemorrhagic activity was tested.

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## MATERIALS AND METHODS

### *Materials*

The lyophilized venoms were obtained from Miami Serpentarium Laboratories and Ross Allen's Reptile Institute. Standard metal solutions were purchased from Beckman Instrument Co., *p*-toluene-sulfonyl-L-arginine methyl ester (TAME), *N*-benzoyl-L-arginine ethyl ester (BAEE), and casein were purchased from Calbiochem, EDTA disodium salt and tris from Fisher Scientific Products. All inorganic compounds were of analytical reagent quality. The water used in making all solutions was triply distilled by a Corning A6-1b glass distillation apparatus.

### *Venoms*

The venoms used in these experiments were obtained in a lyophilized form. *Crotalus atrox* (Western Diamond Back Rattlesnake), *Crotalis basciliscus* (Mexican West Coast Rattlesnake), *Crotalus durissus totonacus* (Totonacan Rattlesnake), *Crotalus horridus horridus* (Timber Rattlesnake), *Crotalus horridus atricaudatus* (Canebrake Rattlesnake), *Sistrurus milarius barbouri* (Dusky Pigmy Rattlesnake), *Bitis arietan* (Puff Adder), and *Bitis gabonica* (Gaboon Viper) were purchased from Miami Serpentarium Laboratories in Miami, Florida. *Crotalus adamanteus* (Eastern Diamond Back Rattlesnake), *Crotalus durissus* (Central American Rattlesnake), *Crotalus durissus terrificus* (South American Tropical Rattlesnake) and *Crotalus viridis viridis* (Praire Rattlesnake), were obtained from Ross Allen Reptile Institute in Silver Springs, Florida. *Agkistrodon acutus* (Hundred-pace Snake), and *Naja naja atra* (Formosan Cobra) were collected by Tu in 1966 and 1967. *Naja naja* (Indian Common Cobra) was sent by the Haffkine Institute in Bombay, India. *Bungarus fasciatus* (Banded Krait) and *Vipera russelli siamensis* (Thailand Russell Viper) were purchased from the Queen Saovabha Memorial Institute in Bangkok, Thailand.

### *Metal analysis*

A 1% solution of each venom was prepared with a 1000 ppm lanthanum oxide solution;* the lanthanum solution is used to prevent the formation of Ca-phosphate complexes which are not dissociated in the Acetylene-air flame. No absorption occurs for the Ca atoms while they are bound in these molecular compounds. The venom samples were then analyzed for metal content on a Beckman Atomic Absorption modular system (model 1301 Atomic Absorption unit with Laminer flow burner-acetylene and air, DB-G Spectrophotometer, and 6 in. chart recorder). Polyethelene containers were used when possible as they contained no detectable levels of metal contaminants. The specific metal concentrations were determined from previously prepared standard curves. A second set of 1% venom solutions was dialyzed against glass distilled water for 48 hr using a dialyzer Model 390 from Instrumentation Specialities Co. The dialysis tubing had been previously treated with EDTA to remove metal ions. Aliquots of the venom solutions were removed at 12 hr intervals; lyopholized and dissolved in the 1000 ppm lanthanum solution. The samples were aspirated into the atomic absorption unit to establish the levels of the remaining metals. The

* 1000 ppm $La_2O_3$ solution—1·18 g $La_2O_3$ in 2 ml conc. HCl with the volume brought to 1 l. with distilled water.

TABLE 1. METAL CONTENTS OF SNAKE VENOMS BEFORE AND AFTER DIALYSIS ANALYZED BY ATOMIC ABSORPTION ($\mu$g metal/g venom)

| Venom (Origin) | Hr* | Ca | Zn | Mg | Na | K | Cu | Mn | Fe | Other metals† |
|---|---|---|---|---|---|---|---|---|---|---|
| Crotalidae | | | | | | | | | | |
| *A. acutus* | 0 | 3000 | 1200 | 450 | 36977 | 1070 | 175 | 0 | 0 | 0 |
| (Formosa) | 48 | 2668 | 522 | 409 | 12780 | 965 | 42 | | | |
| *C. atrox* | 0 | 4196 | 1394 | 701 | 57300 | 410 | 0 | 0 | 0 | 0 |
| (U.S.A.) | 48 | 3780 | 1093 | 344 | 24600 | 320 | | | | |
| *C. adamanteus* | 0 | 1610 | 773 | 107 | 42300 | 750 | 0 | 0 | 0 | 0 |
| (U.S.A.) | 48 | 1604 | 452 | 97 | 8400 | 750 | | | | |
| *C. basiliscus* | 0 | 1989 | 1400 | 376 | 16800 | 670 | 0 | 0 | 0 | 0 |
| (Mexico) | 48 | 1990 | 990 | 310 | 10200 | 638 | | | | |
| *C. durissus* | 0 | 3003 | 1203 | 1470 | 36700 | 13500 | 0 | 0 | 0 | 0 |
| (Central America) | 48 | 2968 | 700 | 775 | 12800 | 3970 | | | | |
| *C. durissus* | 0 | 2390 | 1856 | 342 | 45700 | 1660 | 0 | 0 | 0 | 0 |
| *terrificus* | 48 | 2280 | 1380 | 204 | 1780 | 1440 | | | | |
| (South America) | | | | | | | | | | |
| *C. durissus* | 0 | 1633 | 840 | 117 | 28800 | 590 | 0 | 0 | 0 | 0 |
| *totonacus* | 48 | 1590 | 680 | 100 | 1500 | 550 | | | | |
| (Mexico) | | | | | | | | | | |
| *C. horridus* | 0 | 4930 | 980 | 973 | 53000 | 420 | 0 | 0 | 0 | 0 |
| *horridus* | 48 | 3629 | 800 | 406 | 21900 | 400 | | | | |
| (U.S.A.) | | | | | | | | | | |
| *C. horridus* | 0 | 150 | 680 | 129 | 49900 | 350 | 0 | 0 | 0 | 0 |
| *atricaudatus* | 48 | 97 | 657 | 91 | 10010 | 240 | | | | |
| (U.S.A.) | | | | | | | | | | |
| *C. viridis* | 0 | 4560 | 1847 | 240 | 26400 | 710 | 0 | 0 | 0 | 0 |
| *viridis* | 48 | 2730 | 1050 | 209 | 1200 | 600 | | | | |
| (U.S.A.) | | | | | | | | | | |
| *S. milarius* | 0 | 4000 | 2010 | 446 | 39500 | 2540 | 200 | 0 | 0 | 0 |
| *barbouri* | 48 | 2750 | 1525 | 297 | 1550 | 2159 | 90 | | | |
| (U.S.A.) | | | | | | | | | | |
| Viperdae | | | | | | | | | | |
| *B. arietans* | 0 | 2306 | 1000 | 700 | 41500 | 500 | 0 | 500 | 0 | 0 |
| (South Africa) | 48 | 1200 | 846 | 274 | 500 | 439 | | 52 | | |
| *B. gabonica* | 0 | 2900 | 690 | 636 | 36400 | 220 | 0 | 0 | 0 | 0 |
| (South Africa) | 48 | 1080 | 680 | 277 | 750 | 220 | | | | |
| *V. russelli* | 0 | 1987 | 1800 | 976 | 34100 | 760 | 0 | 0 | 0 | 0 |
| *siamensis* | 48 | 1306 | 809 | 306 | 654 | 310 | | | | |
| (Thailand) | | | | | | | | | | |
| Elapidae | | | | | | | | | | |
| *N. naja* | 0 | 1000 | 1600 | 840 | 60200 | 150 | 0 | 200 | 0 | 0 |
| (India) | 48 | 105 | 360 | 650 | 24800 | 100 | | 521 | | |
| *N. naja* | 0 | 1000 | 380 | 650 | 43600 | 300 | 0 | 13 | 0 | 0 |
| *atra* (Formosa) | 48 | 138 | 170 | 317 | 25250 | 109 | | 3 | | |
| *B. fasciatus* | 0 | 1620 | 196 | 810 | 26500 | 391 | 0 | 0 | 0 | 0 |
| (Thailand) | 48 | 137 | 139 | 500 | 24700 | 110 | | | | |

* Length of time that crude venoms were dialyzed against distilled water before analysis.
† Mo, Bi, Se, Pt, Pd, Ag and Au.

dialyzed venoms were subjected to 0·1 M EDTA for 6 hr, and again dialyzed against distilled water for 36 hr to remove EDTA. The venoms were lyophilized and examined by atomic absorption as before.

*Lethal, hemorrhagic and enzymatic activities*

The toxicity of the crude venom and of the EDTA-treated venom was statistically compared using a modified method of Litchfield and Wilcoxon.[7] The venom samples were dissolved in distilled water and 0·10 ml of the venom solutions were injected intravenously into the tail vein of 20 g Swiss white mice. Ten dose levels of venom were used to determine the toxicity with ten mice being used in each dose. After 24 hr, the number of surviving mice was determined and the lethality ($LD_{50}$) estimated.

Crude venom, EDTA-treated venom, EDTA treated with Mg, EDTA treated with Zn, and EDTA-treated venom with Ca ions were used in the following analyses:

Hemorrhagic activity was estimated using the method of Tu *et al.*[8,9] Swiss white mice weighing 20 g were subcutaneously injected with various amounts of venom (2–20 $\mu$g) in 0·1 ml saline solution. A saline solution (0·1 ml) was injected as a control. The mice were sacrificed after 6 hr; the skins were removed and immediately photographed. The degree of hemorrhage was determined with an Evans Electroselenium Ltd (EEL) "Scanner", by scanning the negative to measure the degree of hemorrhage. The area of the resulting curve was measured with a Gelman Model 30231 Planimeter, Proteolytic activity was followed using a modified method of Kunitz[10] with casein as

TABLE 2. METAL CONTENTS OF SNAKE VENOMS AFTER EDTA TREATMENT AND EFFECT ON LETHALITY

| Venoms | Metal content ($\mu$g metal/g venom) Ca | Mg | Zn | Lethality ($LD_{50}$ $\mu$g/g) before EDTA | after EDTA |
|---|---|---|---|---|---|
| Crotalidae | | | | | |
| *A. acutus* | 0 | 0 | 8 | 0·38 | 0·38 |
| *C. atrox* | 0 | 14 | 86 | 3·56 | 3·57 |
| *C. adamanteus* | 0 | 0 | 100 | 2·36 | 2·41 |
| *C. basiliscus* | 0 | 0 | 51 | | |
| *C. durissus* | 0 | 0 | 3 | | |
| *C. durissus terrificus* | 4 | 0 | 120 | 0·35 | 0·41 |
| *C. durissus totonacus* | 6 | 3 | 18 | | |
| *C. horridus horridus* | 0 | 25 | 200 | 2·57 | 2·47 |
| *C. horridus atricaudatus* | 0 | 10 | 17 | | |
| *S. milarius barbouri* | 15 | 10 | 250 | 12·59 | 12·47 |
| *C. viridis viridis* | 0 | 0 | 200 | 1·01 | 0·99 |
| Viperidae | | | | | |
| *V. russelli siamensis* | 0 | 0 | 3 | 2·11 | 2·01 |
| *B. arietans* | 17 | 13 | 46 | 2·00 | 1·90 |
| *B. gabonica* | 0 | 0 | 33 | 4·95 | 5·2 |
| Elapidae | | | | | |
| *N. naja* | 0 | 200 | 86 | 0·13 | 0·11 |
| *N. naja atra* | 0 | 100 | 80 | 0·29 | 0·30 |
| *B. fasciatus* | 0 | 210 | 100 | | |

the substrate. One ml of a 1% solution of casein buffered at pH 7·0 by 0·1 M phosphate was added to 0·5 ml venom solution. After the mixture was incubated for 30 min at 37°, 2·0 ml of 5% trichloracetic acid was added to stop the reaction. On standing at room temperature for 30 min, the change in absorbance of the supernate was read at 280 mμ on a Beckman DB-G spectrophotometer.

Esterase activities were obtained by measuring the rates of hydrolysis of TAME and BAEE. The method used was that of Toom *et al.*[11]

## RESULTS

### *Metal content*

In order to establish overall metal distribution in snake venoms, the venoms of eleven Crotalidae, three Viperdae, and three Elapidae were analyzed for 15 metals by

TABLE 3. RESTORATION OF HEMORRHAGE ACTIVITY BY METAL IONS*

| Venoms | Venom before EDTA treatment ($mm^2$) | EDTA-treated venom | | | | | |
|---|---|---|---|---|---|---|---|
| | | Addition of metals | | | | | |
| | | No metals | Mg ($mm^2$) | | Zn ($mm^2$) | | Ca ($mm^2$) |
| Crotalidae | | | | | | | |
| *A. acutus* | 11·65 | 0 | 3·09 (26·5%)† | | 0·35 (3%) | | 0 |
| *C. atrox* | 12·41 | 0 | 4·84 (39·0%) | | 0·62 (5%) | | 0 |
| *C. adamanteus* | 11·69 | 0 | 2·47 (21·2%) | | 0·12 (1%) | | 0 |
| *C. basiliscus* | 12·11 | 0 | 1·65 (13·6%) | | 0·66 (6%) | | 0 |
| *C. durissus* | 13·06 | 0 | 1·96 (15·0%) | | 0·42 (4%) | | 0 |
| *C. durissus terrificus* | 9·42 | 0 | 0·97 (10·3%) | | 0·19 (2%) | | 0 |
| *C. durissus totonacus* | 10·25 | 0 | 2·05 (20·0%) | | 0·31 (3%) | | 0 |
| *C. horridus horridus* | 13·48 | 0 | 3·11 (23·1%) | | 0·54 (4%) | | 0 |
| *C. horridus atricaudatus* | 11·95 | 0 | 2·28 (19·1%) | | 0·24 (2%) | | 0 |
| *C. viridis viridis* | 12·34 | 0 | 2·46 (19·9%) | | 0·75 (6·1%) | | 0 |
| *S. milarius barbouri* | 8·05 | 0 | 1·77 | | 0·32 (4%) | | 0 |
| Viperdidae | | | | | | | |
| *V. russelli saimensis* | 2·16 | 0 | 0·54 (25%) | | 0·11 (5%) | | 0 |
| *B. arietans* | 6·41 | 0 | 1·54 (24%) | | 0·46 (7·1%) | | 0 |
| *B. gabonica* | 4·35 | 0 | 1·00 (23%) | | 0·26 (6%) | | 0 |
| Elapidae | | | | | | | |
| *N. naja* | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *N. naja atra* | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *B. fisciatus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* For hemorrhagic test, the skins were removed after venom injection and photographed. Hemorrhagic activity was expressed as the areas under the curve scanned by EEL Scanner on the negative.

† The numbers in parentheses indicated the per cent hemorrhagic activity recovered.

atomic absorption. Metal content of venoms were analyzed by dialyzing venom against water for 12, 24 and 48 hr. Dialysis of 48 hr gave a constant level for each metal. None of the venoms contained detectable amounts of Mo, Bi, Se, Pt, Pd, Ag or Au, while the remaining metals (Ca, Zn, Mg, Na, K, Cu, Mn and Fe) were found in some if not all of the venoms (Table 1). High concentrations of Na and K were found in all venoms. The three most noticeable divalent metals found in the venoms were Ca, Zn and Mg.

*Relation to lethality and hemorrhagic activity*

The atomic absorption analysis of the venoms after treatment with EDTA is given in Table 2. Sodium and potassium were not analyzed as EDTA was added to the venoms as the Na-salt. Ca and Mg concentrations were almost completely removed; Zn remained at about 10 per cent of the original value. The only difference between the families of snakes was that the Mg content remained at a somewhat higher level (30 per cent) in the Elapidae family.

TABLE 4. ESTERASE AND PROTEOLYTIC ENZYME ACTIVITIES OF SNAKE VENOMS BEFORE AND AFTER EDTA TREATMENT

| | Esterase activity | | | | Proteolytic activity* EDTA-treated venoms | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | TAME | | BAEE | | | | Addition of | | |
| Venoms | Before | After | Before | After | No EDTA | No metals | Ca | Zn | Mg |
| Crotalidae | | | | | | | | | |
| *A. acutus* | 2200 | 2350 | 774 | 710 | 21·0 | 0·20 | 1·67 | 3·67 | 2·25 |
| *C. atrox* | 2200 | 2120 | 4030 | 3168 | 10·0 | 0·33 | 1·15 | 2·50 | 3·07 |
| *C. adamanteus* | 7550 | 7000 | 3110 | 3110 | 10·0 | 0·20 | 1·20 | 2·11 | 2·47 |
| *C. basiliscus* | 2120 | 2040 | 32650 | 32610 | 25·0 | 0·35 | 1·71 | 3·74 | 3·12 |
| *C. durissus* | 4940 | 4870 | 3610 | 3550 | 20·0 | 0·25 | 1·19 | 2·67 | 2·00 |
| *C. durissus terrificus* | 2130 | 2220 | 4840 | 4840 | 18·0 | 0·50 | 1·67 | 2·97 | 2·90 |
| *C. durissus totonacus* | 5960 | 5830 | 21380 | 20185 | 15·0 | 0·40 | 1·56 | 3·00 | 3·33 |
| *C. horridus horridus* | 3330 | 3331 | 7100 | 7060 | 16·0 | 0·20 | 2·00 | 2·25 | 2·50 |
| *C. horridus atricaudatus* | 2178 | 2130 | 4930 | 4920 | 20·0 | 0·77 | 1·31 | 2·75 | 3·00 |
| *C. viridis viridis* | 2790 | 3039 | 30370 | 30375 | 17·0 | 0·33 | 2·00 | 3·67 | 3·00 |
| *S. milarius barbouri* | 2140 | 2099 | 3429 | 3449 | 12·0 | 0·20 | 1·17 | 1·25 | 1·69 |
| Viperidae | | | | | | | | | |
| *V. russelli siamensis* | 710 | 675 | 1675 | 1669 | 5·0 | 0·0 | 0·50 | 1·00 | 1·50 |
| *B. arietans* | 909 | 921 | 3690 | 3550 | 10·0 | 0·75 | 0·33 | 1·50 | 1·75 |
| *B. gabonica* | 1770 | 1620 | 5685 | 5580 | 12·0 | 0·33 | 0·50 | 1·50 | 1·50 |

* Proteolytic and esterase activity unit is defined as (absorbance change/min/mg venom) × 1000.

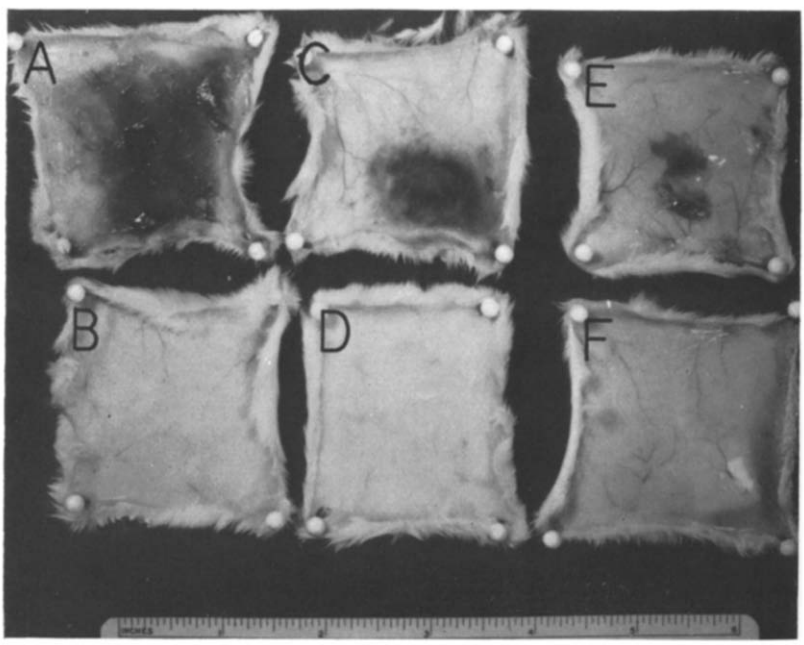


FIG. 1. Inactivation and restoration of venom hemorrhagic activity in *Crotalus atrox*. Hemorrhage caused in mice by: (A) 28 $\mu$g venom (B) 28 $\mu$g EDTA-treated venom (C) 70 $\mu$g EDTA-treated venom with 25 $\mu$g $Mg^{2+}$ (D) 70 $\mu$g EDTA-treated venom with $\mu$g $Ca^{2+}$ (E) 70 $\mu$g EDTA-treated venom with 5 $\mu$g $Zn^{2+}$ (F) Saline injection (0·1 ml).

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Lethality ($LD_{50}$, $\mu g/g$) was determined before and after the addition of EDTA to the venoms; no change was detected. Hemorrhagic activity was completely lost after treatment of the venoms with EDTA (Table 3). The extent of hemorrhage was determined by measuring the intensity and surface area of the damage. For possible regeneration of hemorrhagic activity, Ca, Mg or Zn ions were added to EDTA-treated venom and the amount of hemorrhage was determined as before. Table 3 gives the relative amounts of hemorrhage restored. Calcium ions did not restore hemorrhagic activity, while zinc ions restore a small degree of hemorrhagic activity and magnesium ions restored 10–39 per cent of the original hemorrhagic activity. Figure 1 shows an experiment in the determination of the hemorrhagic activity of *Crotalus atrox*.

*Metal content and enzymatic activities*

Enzymatic activity of protease was measured by following the rate of hydrolysis using casein as the substrate; TAME and BAEE were used for the determination of esterase activity (Table 4). No enzyme activity toward TAME or BAEE was noted in the Elapidae Family as previously reported.[1,2] Table 4 shows high proteolytic activity in the crude venoms whereas only a residual amount of this activity remains after EDTA treatment of the venom. The addition of metal ions (Ca, Mg and Zn) slightly restored the proteolytic activity. Ca ions, as before, were least able to restore proteolytic activity. Mg and Zn ions restored proteolytic activity without any apparent preference to the ions; whereas the restoration of the hemorrhagic activity shows a definite preference for Mg ions over Zn or Ca. The esterase activity of Crotalidae and Viperdae venoms was not affected by the removal of metals.

## DISCUSSION

It has been reported that some metals were found in snake venoms but these studies usually involved only two to five snakes. The information obtained was highly incomplete and randomly selected, therefore, it is not clear whether particular metals found were universally present in all venoms or just in that particular venom.

This report is a systematic investigation of metal contents in venoms of various snakes from wide geographical origins. It was noted that three divalent metals (Ca, Zn, Mg) were found regardless of species. Cu, Fe and Mn were found in several venoms without apparent regard for species. Monovalent metals (K, Na) were found in high concentration in all venoms. It is assumed that they are present as charge balancing ions for the proteins and inorganic salts of the venoms. No direct correlation as to metal content to the species of snake, can be made from this information, however, Crotalidae venoms, i.e. rattlesnake and copperhead contain more Ca and Zn than Elapidae venoms (1600–3700 $\mu g$ Ca and 450–1500 $\mu g$ Zn/g venom for Crotalidae, 1100–1300 $\mu g$ Ca and 680–846 $\mu g$ Zn for Viperdae, and 105–138 $\mu g$ Ca and 139–770 $\mu g$ Zn for Elapidae). Moav *et al*[6] found Zn levels up to 500 $\mu g/g$ in *Viperia Palistenia*; this value corresponds to the concentration of Zn that we found. They also found Cu at a concentration of 1000 $\mu g/g$ venom in *Viperia Palistenia*; however, we could not establish Cu as being distributed in the venoms tested. Only *Akistrodon acutus* and *S. milarius barbouri* contained measurable amounts of Cu.

Of the biological parameters investigated, lethality was not affected by removal of metals, although a striking change in the degree of hemorrhagic activity was noted.

All of the hemorrhagic activity disappeared following the removal of divalent metals from snake venoms. Proteolytic activity was greatly reduced in the EDTA-treated venom while esterase activity was not affected.

Reconstitution experiments show (Table 3) that a 5- to 10-fold increase of the original concentration of Mg or Zn will restore a considerable amount of hemorrhagic activity. Proteolytic activity was also restored by addition of Mg or Zn but to a lesser degree.

An attempt to further clarify the role of metals in the biological activities of the venoms is obviously highly desirable. Isolations and characterizations of pure hemorrhagic toxins are underway at this time to accomplish these objectives.

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