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PRELIMINARY STUDIES ON THE VENOM OF THE CHINESE SNAKE *AZEMIOPS FEAE*, BOULENGER (FEA'S VIPER)

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D. K. VEST. Preliminary studies on the venom of the Chinese snake *Azemiops feae*, Boulenger (Fea's viper). *Toxicon* **24**, 510–513, 1986. — Fea's viper (*Azemiops feae*) produces a venom which is highly toxic to mice when injected by the s.c. or i.v. routes. The i.v. LD_{50} of *Azemiops* venom for Swiss-Webster laboratory mice is 0.52 mg/kg. *Azemiops* venom produces no hemorrhagic activity in mice or rabbits. Immunodiffusion indicates that some fractions of *Azemiops* venom are antigenically related to viperid, elapid and crotalid venoms. SDS-PAGE electrophoresis reveals that this venom contains as many as 22 proteinaceous components.

THE FEA'S VIPER (*Azemiops feae*) is a small viperine snake distributed in the Chinese provinces of Yunan, Guizhou, Sichuan, Guangxi, Fujian, Jiangxi and Zhejiang (ZHAO and ZHAO, 1981) and also occurs in Burma. Due to the rarity of this serpent, virtually nothing is known concerning its venom. Histological investigation of *Azemiops* venom glands by KOCHVA and GANS (1965, 1966) and KOCHVA *et al.* (1967) show that *Azemiops* venom glands exhibit the general pattern of taxonomic characteristics of all viperine snakes.

Seven adult specimens of *Azemiops feae* were obtained from a commercial source (J. J. Vandenbrink, Jabria B.V.) and were subjected to venom extraction. The venom was collected in a small plastic 'auto-analysis' cup (Sardstedt no. 73.641), immediately frozen, then lyophilized and weighed. Lyophilized venom was reconstituted in 0.9% physiological saline. Twenty-five healthy Swiss-Webster laboratory mice weighing 18–22 g were used for the lethality screen. The i.v. LD_{50} was calculated according to the method of LITCHFIELD and WILCOXON (1949). All mice were closely observed for 12 hr post-challenge. Respiratory rates were measured by counting respirations against a lap timer. Thirteen Swiss-Webster mice were depilated and challenged s.c. with venom concentrations of 5–50 μg in 100 μl 0.9% physiological saline. Additionally, a California giant laboratory rabbit was dorsally depilated and challenged intracutaneously with six concentrations of (5–50 μg) *Azemiops* venom reconstituted as described above. Mice surviving s.c. challenge, as well as the rabbit, were killed at 24 hr post-injection and examined for hemorrhagic and local responses. Immunodiffusion of *Azemiops* venom against several commercial monovalent and polyvalent antivenoms, as well as active monovalent antisera of rabbit origin (Arizona State University), was performed on Ouchterlony plates and micro-immunodiffusion slides using 2% agar noble in the

diffusion medium. Antigen wells were loaded with venom solutions containing 5 mg/ml *Azemiops* venom, and were developed at room temperature. SDS-Polyacrylamide gel electrophoresis (slab format) was performed at pH 6.8 according to the method of LAEMMLI (1970). Wells were loaded in duplicate with venom quantities of 50, 75, 100, 125 and 150 μg. All samples were reduced with β-mercaptoethanol and run at 10 mA per gel through a 3 mm thick, 5% stacking gel, then a 15% separating gel.

Five of the *Azemiops* specimens subjected to extraction delivered only trace amounts of venom, despite vigorous biting. The other two specimens delivered significant, approximately equivalent volumes of venom (~1.75 mg/snake). The venom appeared as a clear, golden-yellow liquid, similar to that of many other viperine snakes. Its viscosity, likewise, was similar to that delivered by most viperines.

The i.v. LD_{50} of *Azemiops feae* venom in Swiss-Webster mice was 0.52 mg/kg. Mice challenged with doses of 0.50 – 0.60 mg/kg usually exhibited a transitory, minor-to-moderate vasodilatation of ear vessels within 10 min of injection. These mice became torpid within 30 ± 10 min and respirations began to decrease, generally followed by clonic convulsions. Paralysis became virtually complete 90 – 110 min post-injection, although mice retained a very slight ability to move the legs and were able to right themselves until the very terminal stage of poisoning. A paroxysm occurred 86 – 150 min post-injection, in which respirations reached a critical minimum (less than 35 per min). This paroxysm terminated in death for mice challenged by a lethal dose; all mice that tolerated envenomation for 180 min survived. All mice receiving s.c. challenges of between 0.50 and 0.60 mg/kg died, while those receiving 0.40 mg/kg or less survived.

Local tissue responses and hemorrhagic activity in laboratory mice were only slight. No indications of frank hemorrhagic activity nor local tissue degradation were evident. Intracutaneous injection of *Azemiops* venom into rabbit skin produced no local manifestations or evidence of hemorrhagic phenomenon.

Ouchterlony and micro-immunodiffusion of *Azemiops* venom vs. commercial antivenins resulted in the formation of precipitation lines between wells containing *Azemiops* venom and several commercial antivenins (Fig. 1). Significant precipitation lines developed against the antisera to tiger-snake, death adder, mamba and Iran cobra, while weak reactions were seen against Thai cobra, and no reaction developed against king cobra. Strong, multiple precipitation lines developed against Serum Europe, Serum North Africa, Fitzsimmon's Polyvalent and Wyeth Polyvalent, with weaker lines visible against Haffkine Polyvalent and Serum Near and Middle East. No precipitation reaction was observed against monovalent *Agkistrodon acutus* antivenene or Malayan pit viper antivenene, nor against monovalent *Agkistrodon bilineatus*, *Agkistrodon piscivorus*, *Crotalus atrox*, *Crotalus scutulatus* or *Heloderma suspectum* antisera.

SDS-PAGE of β-mercaptoethanol-reduced *Azemiops* venom revealed the presence of 22 visible proteinaceous bands with molecular weights ranging from approximately 10,000 to 80,000. When 100 – 150 μg of venom were applied, 20 – 22 bands were visible, including one smaller component (mol. wt 9000 – 11,000) not well visualized when lesser amounts of venom were applied.

Fea's viper (*Azemiops feae*) produces a venom which causes marked flaccid paralysis, respiratory depression and clonic convulsions in mice, while eliciting only negligable local tissue responses. There was no apparent hemorrhagic phenomenon, either local or systemic. Generally, convulsions signaled the onset of a paroxysm in which respiratory rates fell below 30 per min and finally ceased altogether, terminating in the death of the mouse. Superficially, the progressive respiratory depression, which closely paralleled the

progression of flaccid paralysis, resembles poisoning by those elapid snakes whose venoms contain a post-synaptic, peptide neurotoxin.

The present study using mice indicates that *Azemiops* envenomation may be expected to produce visual, respiratory and paralytic effects in humans, but does not suggest that this venom is capable of inducing local or hemorrhagic phenomenon, as reported for humans (ZHAO and ZHAO, 1981).

Immunodiffusion of *Azemiops* venom against multi-genus polyvalent antivenins demonstrate the presence of antigenically related components between *Azemiops* and all major groups of terrestrial venomous snakes. Precipitation lines between *Azemiops* venom and monovalent Australian antivenenes, as well as genus-specific mamba antivenene, indicate a definite antigenic relationship between these venoms and *Azemiops*, but it should be noted that these sera react with an exceptionally large number of heterologous venoms, including colubrid Duvernoy's secretions (MINTON, 1979; Minton, personal communication). Wyeth Polyvalent Antivenin proved to be the only crotalid preparation reacting against *Azemiops* venom, with no reaction seen against monovalent *Agkistrodon* or *Crotalus* antisera.

Azemiops feae may be considered a member of the Viperidae which possesses a venom with immunological and electrophoretic characteristics similar to those of other viperine

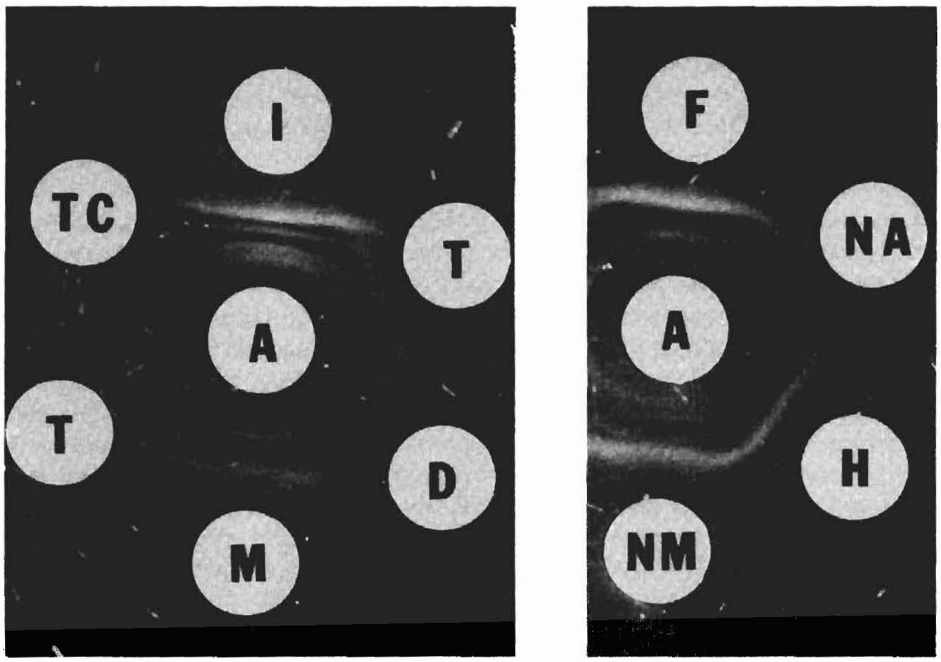

FIG. 1. MACRO OUCHTERLONY IMMUNODIFFUSION OF SOME ANTIVENINS ACTIVELY PRECIPITATING AGAINST *Azemiops feae* VENOM.

Photographed 72 hr post-loading: (A) *Azemiops feae* venom, 5 mg/ml; (D) death adder antivenom; (F) Fitzsimmon's Polyvalent; (H) Haffkine Polyvalent; (I) Iran cobra antivenom; (M) mamba Polyvalent; (T) tiger snake antivenom; (NA) Serum North Africa; (NM) Serum Near and Middle East; (TC) Thai cobra antivenom. Comparable, but more sharply defined precipitations were observed using micro-immunodiffusion slides.

snakes. Its toxicity characteristics, however, are not typical of the majority of the better known, clinically significant Viperidae, whose venoms induce changes in local tissues, hemorrhage and hematological discrasias. *Azemiops* venom appears to be one of the 'atypical' viperid venoms, which, like the berg adder *Bitis atropos* (CHRISTENSEN, 1955, 1968), elicits pronounced paralytic phenomenon in mice, without evidence of local and/or hemorrhagic syndromes.

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REFERENCES

CHRISTENSEN, P. A. (1955) *South African Snake Venoms and Antivenoms.* Johannesburg: South African Institute of Medical Research.

CHRISTENSEN, P. A. (1968) Venoms of Central and South African snakes. In: *Venomous Animals and their Venoms*, Vol. 1, p. 447 (BÜCHERL, W., BUCKLEY, E. and DEULOFEU, V., Eds). New York: Academic Press.

KOCHVA, E. and GANS, C. (1965) The venom gland of *Vipera palaestinae* with comments on the glands of some other Viperinae. *Acta Anat.* **62**, 365.

KOCHVA, E. and GANS, C. (1966) Histology and histochemistry of the venom gland of some crotaline snakes. *Copeia* **3**, 506.

KOCHVA, E., SHAYER-WOLLBERG, M. and SOBOL, R. (1967) The special pattern of the venom gland in *Atractaspis* and its bearing on the taxonomic status of the genus. *Copeia* **4**, 763.

LAEMMLI, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680.

LITCHFIELD, J. T. JR and WILCOXON, I. (1949) A simplified method of evaluating dose–effect experiments. *J. Pharmac. exp. Ther.* **96**, 99.

MINTON, S. A. (1979) Common antigens in snake venoms. In: *Handbook of Experimental Pharmacology*, Vol. 52, *Snake Venoms*, p. 847 (Lee, C.-Y., Ed.). New York: Springer-Verlag.

ZHAO, E. and ZHAO, G. (1981) Notes on Fea's viper (*Azemiops feae* Boulenger) from China. *Acta herpetol. sin.* **5**, 71.