J. M. Yadlowski,[1] B.S.; A. T. Tu,[1] Ph.D.; J. C. Garriott,[2] Ph.D.; and L. E. Norton,[2] M.D.

Suicide by Snake Venom Injection

REFERENCE: Yadlowski, J. M., Tu, A. T., Garriott, J. C., and Norton, L. E., "**Suicide by Snake Venom Injection,**" *Journal of Forensic Sciences,* JFSCA, Vol. 25, No. 4, Oct. 1980, pp. 760–764.

ABSTRACT: A case of apparent suicide by injection of snake venom was investigated. Evidence found at the scene and presumably used for self-injection was positive for nonspecific venom but had the characteristics of a viper venom.

KEY WORDS: toxicology, suicide, venoms

Suicide by injection of snake venom is rare and seems to be associated with some previous experience with snakes or some special knowledge of the effects of envenomization. Documented cases of suicide by this method have appeared in the recent literature [*1,2*]. The following case report is that of an apparent suicide by snake venom injection.

Immunodiffusion, isotachophoresis,[3] toxicity testing, and spectrophotometric studies were used to link the syringe found at the scene to snake venom and to the death of the victim.

Case Report

A 23-year-old employee of a snake farm was found dead in a wooded area 24 h after he disappeared. A number of suicide notes were left behind. Two vials, coated on the inside with a white crystalline powder, and a syringe were found near the body. Presumably, the material in the vials was mixed with water and used to fill the syringe for injection. It was postulated that the victim killed himself by injection of snake venom because of his knowledge of and access to snake venoms. The deceased had threatened to commit suicide by this means.

Postmortem examination revealed a recent injection site on the back of the left hand, near the base of the third finger, which was swollen and hemorrhagic. Except for moderate decomposition, there was no other significant finding.

This investigation was in part supported by National Institutes of Health Grant 2R01 GM15591. Received for publication 25 April 1980; accepted for publication 9 May 1980.

[1]Graduate student and professor, respectively, Department of Biochemistry, Colorado State University, Fort Collins.

[2]Chief toxicologist and medical examiner, respectively, Southwestern Institute of Forensic Sciences, Dallas, Tex.

[3]Technique to separate components by constant current electrophoresis.

Copyright © 1980 by ASTM International

Methods

The materials analyzed consisted of two vials of white powder, residue from the syringe and needle, blood from the victim, and a saline extract of tissue from the injection site.

Ultraviolet spectrophotometry was performed on the powder and syringe residue to determine if protein was present. Immunodiffusion studies on all samples were performed with multipolar antivenins. The antivenins used for immunodiffusion assays were these:

(1) South Africa antivenin (manufactured by South African Institute for Medical Research, Johannesburg, S. Africa) for *Naja nivea* (Cape cobra), *Haemachates haemachatus* (ringhals), *Bitis arietans* (puff adder), and *Bitis gabonica* (Gaboon adder);

(2) Central Africa antivenin (manufactured by Beringwerke, Marburg, Germany) for *Bitis* species, *Dendraospis* species, *Haemachates* species, and *Naja* species;

(3) Middle East antivenins (manufactured by Beringwerke, Marburg, Germany) for *Cerastes* species, *Echis* species, *Naja* species, and *Vipera* species; and

(4) Thailand antivenin (manufactured by Saobabha Memorial Institute Bangkok, Thailand) for *Naja naja siamensis* (Thailand cobra).

Immunodiffusion tests were performed by the method described previously [*3*].

The potency of the antivenins was checked against venoms from *Naja naja siamensis*, *Naja haje*, *Naja nigricollis*, *Ophiophagus hannah*, *Naja naja*, and *Naja melanoleuca*. Isotachophoresis and toxicity studies were performed on all the samples.

No mean lethal dose (LD_{50}) value was measured because there was insufficient quantity of sample. Toxicity was tested by intravenous injection of three 20-g Swiss Webster mice. The injection volume was 0.1 mL, and the animals were observed for 24 h.

Capillary isotachophoresis was performed on an LKB (Uppsala, Sweden) Tachophor® Model 2127 using both anionic and cationic systems [*4*]. In the anionic system, chloride was used as the leading ion at pH 9.0, 0.01*M*, and ε-aminocaproic acid as the trailing ion at pH 10.5, 0.01*M*. The cationic system used cacodylic acid as the leading ion at pH 7.0, 0.0048*M*, with creatinine as the trailing ion at pH 5.1, 0.01*M*. Both anionic and cationic systems used 2 μL of a 1% ampholine solution (pH 3.5 to 10) to provide a mobility spacing gradient.

Results

The summary of tests is given in Table 1.

Immunodiffusion using multipolar antivenins from Central Africa (Fig. 1, II), South Africa (Fig. 1, IV), and the Middle East (Fig. 1, III) was positive for snake venom on the contents of the syringe and material on the needle. Antivenin against Thailand cobra (*Naja naja siamensis*) (Fig. 1, I) was negative. The precipitin patterns of these antivenins with known samples of cobra venoms (not shown) were obtained for comparison with the unknown venom (the contents of the syringe) and as a check on the antivenins (Fig. 1). South African antivenin produced a dark distinct line (Fig. 1, IV). Central African antivenin produced a double precipitin line with the unknown material (Fig. 1, II).

The contents of the vials gave no immunoprecipitin reaction with all dilutions of antivenin, indicating that the material in the vials was not snake venom. The immunodiffusion

TABLE 1—*Summary of tests.*

Material	Test System	Result
Powder on top of needle	immunodiffusion	negative against Thailand cobra antivenin; positive against polyvalent antivenin for Viperidae and Elapidae
	toxicity	severe hemorrhage in mice
Powder inside syringe	immunodiffusion	same result as above
	toxicity	severe hemorrhage in mice; one out of three mice died
	ultraviolet absorption	absorption maxima at 250 and 290 nm
	isotachophoresis	profile resembles a mixture of proteins
Powder in vials	immunodiffusion	negative results
	toxicity	nontoxic, nonhemorrhagic
	ultraviolet absorption	absorption maximum at 290 nm

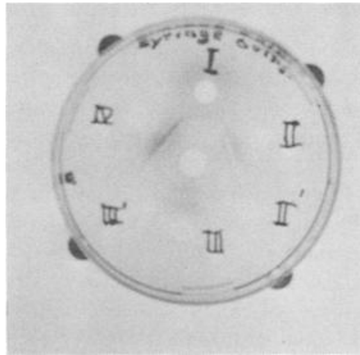

FIG. 1—*Immunodiffusion plate with fluid from syringe in center well and multipolar antivenins from* (I) anti-Naja naja siamensis; (II) *Central African antivenin;* (II^1) *1:10 dilution of II;* (III) *near Middle East antivenin;* (III^1) *1:10 dilution of III; and* (IV) *South African antivenin. Pattern was the same as material from inside the syringe except the lines were darker.*

studies on a sample of the victim's blood and on a saline extract of the tissue were also negative. This was not surprising, considering the blood dilution, the presence of putrefaction, and the sensitivity limits of the method.

Toxicity tests with mice were carried out with contents of the syringe and needle. Three mice, 18 to 22 g, were injected with 0.1 mL of the contents of the syringe and needle that had been dissolved in 1 mL of physiological saline. The material from inside the syringe proved lethal to one of the mice. Both this material and that scraped from the outside of the needle produced hemorrhage in all the mice tested. Since cobra venom usually does not produce hemorrhage [5], it would appear that the venom is not that of a cobra, but that of a viper.

Ultraviolet spectrophotometry of the fluid in the syringe and dissolved material in the vials gave the spectra shown in Fig. 2. These spectra demonstrated two similar maxima (260 and 280 nm), providing evidence that they may represent similar material. Spectrum 1 in Fig. 2 is not representative of most proteins, including snake venoms, which normally have only one maximum at 280 nm. It may be that the unknown sample from the syringe contains other compounds in addition to venom, that is, the material from the vials, or that there was

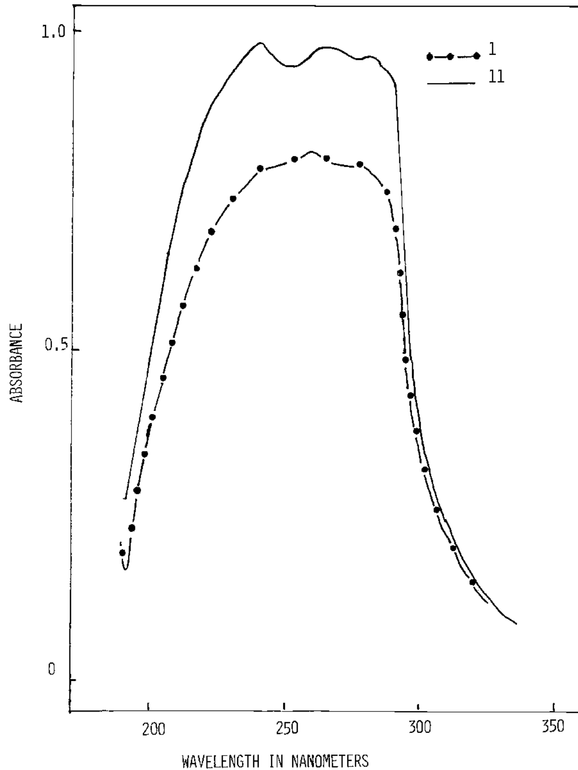

FIG. 2—*Ultraviolet absorption spectra of material examined:* (1) *material in vial and* (II) *material in syringe.*

extensive decomposition of the venom between the time of administration and the time of analysis.

The studies on the material in the syringe using isotachophoresis with a cationic system gave profiles of materials absorbing at 280 nm. The peaks coming off first look similar to the isotachophoresis profile of material from the vials; however, these peaks were not characteristic of proteinaceous material, while the remainder of the profile was quite characteristic of proteins.

Discussion

The evidence showed that a component of the material in the syringe was not similar to the material in the vials. The latter material was much too homogeneous to be a snake venom, as shown by isotachophoresis, and its ultraviolet spectrum showed that it was not a pure protein toxin. Immunodiffusion studies showed it was not snake venom or any purified toxin component that gives an immunoprecipitin reaction with the multipolar antivenins used in the study. The lack of lethal toxicity at the concentrations tested suggested a secondary role in the suicide for the material in the vials. The apparent presence of the vial contents in the syringe suggests that it was used together with the snake venom for making the solution that was injected.

Conclusive evidence was obtained for the presence of snake venom in the syringe and needle, suggesting the use of snake venom as the agent for suicide. More tests using different

monovalent antivenins would have been necessary to identify the exact species of snake. Many commercial antivenins are polyvalent and it is not possible to identify the exact species.

Immunodiffusion studies showed positive precipitation lines against the antivenins of South and Central African snakes, consisting of Elapidae and Viperidae. There was no such line observed against the antivenin of Thailand cobra (*Naja naja siamensis*); therefore, the presence of Thailand cobra venom in the syringe can be definitely excluded.

It was shown previously by one of the authors that *Naja naja atra* venom is almost identical to *Naja naja siamensis* venom and the former has cross-reactivity with the latter antivenins [3]. Therefore the use of Taiwan cobra (*Naja naja atra*) venom and Indian cobra (*Naja naja*) venom can be excluded. Most African cobra (*Naja* and *Haemachates* species) venoms can also be excluded because usually cobra venoms from different localities have immunological cross-reactions. Moreover, injection of the material present in the syringe and needle caused severe hemorrhage in mice at the site of injection. Usually cobra and Elapidae venoms do not show such hemorrhagic activity [5]. Venoms of Viperidae and Crotalidae usually show such hemorrhage after injection. Crotalidae venoms do not cross immunologically against the African antivenins. Thus, the possibility of Crotalidae venoms being present is excluded. Normally Elapidae venoms contain a neurotoxin that causes paralysis in animals on envenomization.

The injection of the material obtained from the vials did not produce hemorrhagic symptoms.

From all this evidence, it is reasonable to assume the white substance inside the syringe and outside the needle is a viper venom of African origin, although the exact species is not identified.

References

[1] Eaker, D. K., Karlson, E., Lic, F., Rammer, L., and Saldeen, T., "Isolation of Neurotoxin in a Case of Fatal Cobra Bite," *Journal of Forensic Medicine*, Vol. 16, No. 3, 1969, pp. 96-99.

[2] Knight, B., Barclay, A., and Mann, R., "Suicide by Injection of Snake Venom," *Forensic Science*, Vol. 10, No. 2, March/April 1977, pp. 141-145.

[3] Tu, A. T. and Ganthavorn, S., "Comparison of *Naja naja siamensis* and *Naja naja atra* Venoms," *Toxicon*, Vol. 5, No. 3, Feb. 1968, pp. 207-211.

[4] Hendon, R. A. and Tu, A. T., "The Role of Crotoxin Subunits in Tropical Rattlesnake Neurotoxic Action," *Biochimica Biophysica Acta*, Vol. 578, May 1979, pp. 243-252.

[5] Tu, A. T., Toom, P. M., and Ganthavorn, S., "Hemorrhagic and Proteolytic Activities of Thailand Snake Venoms," *Biochemical Pharmacology*, Vol. 16, No. 11, 1967, pp. 2125-2130.

Address requests for reprints or additional information to
A. T. Tu, Ph.D.
Department of Biochemistry
Colorado State University
Fort Collins, Colo. 80523