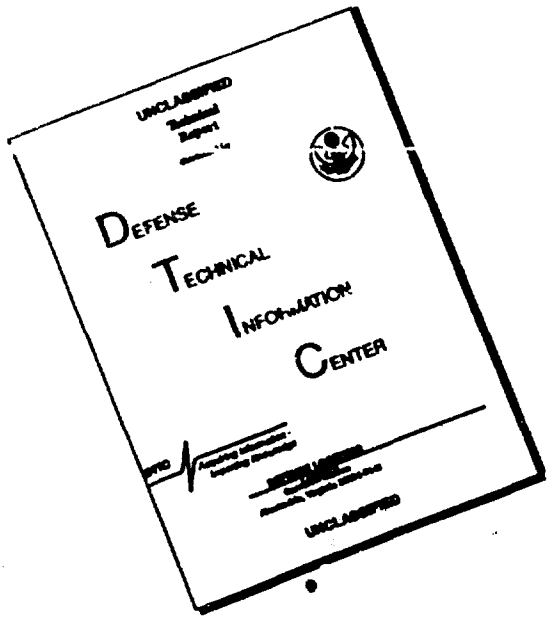
A STUDY ON THE PROPHYLACTIC TOXOID AGAINST VENOM OF ASIAN SNAKES

Yoshio Sawai

Japan Snake Center
Yabuzuka, Japan

July 1968

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

AD

REPORT No. FE-356-2 (Final Report)

CONTRACT No. DAJB17-67-C-0010

DA Project/Task Area/Work Unit No. 3A014501B71P 00 102FE

A STUDY ON THE PROPHYLACTIC TOXOID AGAINST VENOM OF CERTAIN ASIAN SNAKES

by

Yoshio Sawai, D.MSC.

Chief, Laboratory of Snake Venom
Japan Snake Center
Yabuzuka-honmachi, Nittagun,
Gunma-ken, Japan

July 1968

U. S. ARMY RESEARCH AND DEVELOPMENT GROUP
FAR EAST
APO San Francisco 96343

DDC DISTRIBUTION AND AVAILABILITY NOTICE

This document has been approved for public release and sale; its distribution is unlimited.

DISPOSITION INSTRUCTIONS

Destroy this report when it is no longer needed. Do not return it to the originator.

[redacted]

REPORT NO. FE-356-2 (Final Report)
Contract No. DAJB17-67-C-0610
DA Project/Task Area/Work Unit No. 3A014501B71P 00 102FE

A STUDY ON THE PROPHYLACTIC TOXOID AGAINST VENOM OF ASIAN SNAKES

by

Yoshio Sawai, D. MSC
Chief, Laboratory of Snake Venom
Japan Snake Center, Yabuzuka
Nittagun, Gunmaken, Japan

July, 1968

U.S. ARMY RESEARCH AND DEVELOPMENT GROUP
FAR EAST
APO San Francisco 96343

Table of Content

ABSTRACT

The purpose of this study is to help the medical treatment of severe poisonous snake-bite, because there would be a limit in serum treatment of patient who received a large amount of venom. To solute this problem, the writer accumulated data on prophylactic immunization against habu snake venom which is common in South West Islands of Japan.

This report concerns an attempt to immunize experimental animals with Taiwanese snake venoms which were inactivated by dihydrothioctic acid. Reports on surveys of field trial of prophylactic shots with toxoided venom in habu-infested area is also added here.

Five kinds of venom were collected, milked and freezedried from snakes at Taipei of Taiwan. At first, two kinds of Agkistrodon acutus and Bungarus multicinctus were inactivated by dihydrothioctic acid and injected into rabbits and mice. Those immunized animals were challenged intramuscularly by each kind of untreated venom for the test of antigenicity. Blood levels of antibody of immunized animals were also investigated.

The results showed that hemorrhagic effects of venom of A. acutus were prevented in considerable degree in immunized rabbits, although antibody of sera of immunized animals were not so high as antivenin for treatment. Antilethal activity of mice which were treated by venom toxoid of B. multicinctus increased definitely.

In the next, immunological relationship of venom of T. mucrosquamatus, T. stejnegri and T. elegans were investigated. Rabbits were immunized with each of those venom toxoid, and then crossly challenged by those venom respectively. The results indicated that those venoms were immunologically correlated with each other.

Finally, polyvalent toxoid of venom of T. mucrosquamatus, T. stejnegri and A. acutus was also proved to be good antigenic in rabbits by the same method.

Analysis of results of current immunization program from 1965 to 1967 suggested that victims who received toxoided venom previously recovered without severe local lesion. Those results encourage us to investigate effective snake venom toxoid as an aid for medical treatment of severe snakebites.

BLANK PAGE

Introduction

Sawai et al (1961, 1963, 1966) previously reported preparation of freeze-dried antivenin sera against habu (Trimeresurus flavoviridis) venom and its clinical use combined with EDTA, Glycyrrhizine, Tetracycline and Dihydrothioctic acid which were shown useful antidotes for habu-bites.

In spite of the efforts to strengthen the medical treatment of habu-bite the results of the survey on snake-bites on Amami and Ryukyu Islands showed that severe necrosis of lethal cases were still remained in 6 to 10 per cent a year.

As the results of clinical analysis of those severe cases, it was concluded that the severe cases occurred not from inadequate or delayed treatment but from larger amount of venom which were injected into the victims. The amount of venom are so large that intoxication develope rapidly before the victims receive medical treatment. Therefore, it is clear that there is a limit in serum treatment of habu-bites.

In such a situation, prophylaxis against snake venom toxoid seems to be useful not only to gain time for treatment but also remove the fear against severe snake-bite which might down the desire of the residents to work.

Sawai et al (1963) reported inactivation of venom of the habu by dihydrothioctic acid. Toxoided venom did not lose antigenicity, and did not produce any side reactions when injected into experimental animals (Sawai et al. 1966). Following laboratory studies a program was started to attempt to immunize residents of habu-infested areas on Amami and Ryukyu Islands against habu venom (Sawai et al. 1966). Over 25,000 persons received injection of toxoided venom during 1965 and 1966. The program is being continued.

Although most of the studies of the effects of DHTA on venoms were conducted with venom of the habu and mamushi (Agkistrodon halys), Sawai, Kawamura, Fukuyama and Keegan (1967) made tests of the effectiveness of this substance in inactivating toxic effects of other snakes of families Crotalidae, Viperidae and Elapidae.

Purpose of research proposed would be to determine feasibility of active immunization of man against venoms of certain Asian snakes.

This report concern the laboratory studies of the effectiveness in protecting experimental animals against venom of Agkistrodon acutus, Trimeresurus mucrosquamatus Trimeresurus stejnegri, Trimeresurus elegans and Bungarus multicinctus, and analysis of results of the current immunization program against habu venom now being conducted in the Amami and Ryukyu Islands.

Materials and Methods

1. Snake venoms: Taiwanese snake venoms are collected in Taipei on July, 1967 and freeze-dried by the aid of Laboratory of NAMRU 2 in Taipei. Venom of T. elegans was supplied from the Ryukyu Hygiene Laboratory.

2. Dihydrothioctic acid is supplied from Fujisawa Pharmaceutical Co., Osaka.

3. Inactivation of venom: The same amount of venom and DHTA are mixed together and allowed to stand for one hour at 37 degree C.

4. Antigenicity tests

a. Rabbits are injected subcutaneously with 2.5 mg of DHTA treated venom of A. acutus, T. mucrosquamatus, T. stejnegri and T. elegans in amount of 0.5 ml. Three weeks after the first injection, the same dose of 3 booster are injected subcutaneously at the interval of one week. One week after the last booster, 1.2 mg, 2.4 mg and 4.8 mg of untreated venom dissolved in 0.2 ml of saline are challenged intramuscularly into the thigh of immunized and unimmunized rabbits. 24 hours after the challenge, rabbits are sacrificed, skins of the legs are cut off and the muscle are incised and observed presence or absence of hemorrhage or necrosis. Degrees of local lesion are indicated as follows: Swelling -- + slight swelling with serous exudate, ++ marked swelling with hemorrhagic exudate, +++ strong swelling with hemorrhagic exudate. Hemorrhage -- + hemorrhage of pink or red and localized around the site of injection. ++ hemorrhage of red or darkred reached whole the thigh, +++ hemorrhage reach abdominal side. Size of necrosis is inspected by cutting muscles.

b. In mice, 0.05 mg of toxoided venom of B. multicinctus in 0.1 ml is used as the first dose and the first booster dose, and 0.1 mg of toxoided venom is used as second and third booster. 0.2 mg of toxoided venom is used as forth booster.

Just before and one week after the 4th booster, mice are challenged with varying amount of untreated venom ranging from 2.3γ to 75γ.

c. Measurement of antibody in immunized animals

(1) Intramuscular method: 0.1 ml of rabbit serum or pooled sera of mice and 0.1 ml of varying dose of venom are mixed

and incubated at 37 degree C for one hour, and then injected intramuscularly into the leg of mice. 24 hours after the injection, the local lesions and death or survival are observed. Mice are sacrificed and skin of the legs are cut off and the presence or absence of hemorrhage is observed. Degree of local lesion are indicated as follows: 0 -- no lesion or hemorrhage at the point of injection. 1 -- hemorrhage in one third of the thigh. 2 -- hemorrhage in two third of the thigh. 3 -- hemorrhage reach the trunk. And then calculated the mean score of lesions of mice tested. In controls, 0.2 ml of untreated venom are used, and hemorrhage which is calculated as 1 is fixed as minimum hemorrhagic dose (mhd). Antibody level is represented by multiple of mhd which is neutralized by 0.1 ml of serum.

(2) *Intracutaneous method:* Immune serum and venom mixtures as shown above are injected intracutaneously into rabbits which are previously depillated. 24 hours after the injection, rabbits are sacrificed and the skin are taken off and sticked on the glass plate. Hemorrhagic lesions are observed reversing the glass plate, and the degrees of lesions are recorded. Minimum hemorrhagic dose is indicated as hemorrhage of diameter of one cm.

5. Survey of the prognosis of habu-bites: Protocols available from the Public Health Center of Amami and Ryukyu Islands or from the doctors who treated the victims were first investigated. The doctors or victims were asked to tell details of snake-bites to us. The protocols are inspected with emphasis of on the patient who participated to the program of immunization of the habu venom toxoid or not, and who developed severe systemic reaction such as nausea or vomitting, hypotension, cyanosis, diarrhea and abdominal pain, and necrosis of muscles either widespread or localized, deformity or ankylosis.

Results

1. Venom yield of Taiwanese snakes. 5 species of Taiwanese snakes, Agkistrodon acutus, Naja n, atra, Bungarus multicinctus, Trimeresurus mucrosquamatus and Trimeresurus gramineus stejnegri were collected, and their venom were milked and freeze dried. Yield of each venom is indicated in table 1. Yield of each venom varied by each pool of venom. Thus, mean value of yield of venom milked from N. n. atra in a pool was 146.3 mg, whereas 55.1 mg was in another one. Mean yield from B. multicinctus was only 1.7 mg or 1.4 mg.

2. Antigenicity of DHTA treated venom.

a. Venom of Agkistrodon acutus: 25 rabbits weighing 3 kg were immunized with the toxoided venom of A. acutus. There were no signs of hemorrhage or intoxication in rabbits immunized with toxoided venom. One week after the last booster, every 3 rabbits of 3 groups were challenged with 1.2 mg, 2.4 mg and 4.8 mg of the venom respectively. As indicated in table 2, 3 immunized rabbits which were challenged with 1.2 mg showed no detectable lesion, whereas marked hemorrhage were seen in 2 unimmunized rabbits and marked necrosis was observed in one of them. In one of 3 immunized rabbits which were challenged with 2.4 mg of venom no hemorrhage was found. Necrosis was prevented in 2 of the three, although localized necrosis was seen in another one, whereas hemorrhage and definite necrosis were seen in two control rabbits. In another group of immunized rabbits which were challenged with 4.8 mg of venom, necrosis was prevented completely in two rabbits, although slight necrosis was seen in another rabbit whereas severe necrosis occurred in two control rabbits.

b. Antibody level of rabbits immunized with toxoided venom of A. acutus

(1) Antibody level measured by intracutaneous method from rabbits one week after the first and second booster: 0.1 ml of sera taken from 5 rabbits at the interval of a week were mixed with 0.1 ml of varying amount of venom ranging from 0.5γ to 150γ, incubated at 37 degree C for one hour, and then injected intracutaneously into rabbits.

As shown in table 3, antibody levels measured at the interval of one week improved and 0.1 ml of sera neutralized 9.3γ or 37.5γ of venom, whereas 0.5γ of venom showed marked hemorrhage of 7 mm of diameter.

(2) Antibody level after the 3rd booster: Sera taken from 8 rabbits were tested by intramuscular and intracutaneous method. As shown in table 4, 0.5γ of venom induced definite hemorrhage in normal rabbits by intramuscular method, whereas 4.6γ or 18.7γ of venom were neutralized by 0.1 ml of immune sera. In intracutaneous method, 9.3γ or 18.7γ of venom were neutralized by 0.1 ml of immune sera, whereas 1.1γ of venom induced hemorrhage of 8 mm of diameter.

Table 1. Venom yield of Taiwanese snakes

Name of Snakes	Number of Snakes	Venom milked (g)	Venom dried (g)	Venom yield (mg)
A. acutus	16	8.7	1.8186	113.7
N. n. atra	10	5.3	1.4613	146.3
	12	-	0.6617	55.1
T. mucrosq.	4	0.4	0.0563	14.0
	13	-	0.5575	42.9
T. stejnegri	43	-	0.2727	6.3
B. multicinc.	92	1.2	0.1631	1.7
	226	-	0.3133	1.4

Table 2. Antitoxic effects in rabbits immunized with toxoided venom of A. acutus

Challenged Venom	No. of Rabbits	Edema	Hemorrhage	Necrosis
1.2 mg	3	(-)	(-)	0
	18	(-)	(-)	0
	20	(-)	(-)	0
	25	(-)	+	15 x 10x 10
	26	(-)	+	0
2.4 mg	14	+	+	20x 8 x 4
	15	+	(-)	0
	16	+	+	0
	27	+	+	30x 20x 19
	28	+	+	20x 15 x 10
4.8 mg	5	+	+	0
	12	+	+	0
	13	+	+	18 x 13 x 8
	29	+	+	25 x 18 x 10
	30	+	+	35 x 20x 10

Rabbits from No. 25 to No. 30 were used as control. Figure in necrosis indicated the size of lesion in mm.

Table 3. Antibody levels measured by intracutaneous method.

Rabbit Venom (γ)	1		4		6		8		21		24		Untreated
0.5	-		-		-		-		-		-		7 mm
1.1	-		-		-		-		-		-		8
2.3	-		-		-		-		-		-		9
4.6	-		-		-		-		-		-		11
	4W	5W	4W	5W	4W	5W	4W	5W	4W	5W	4W	5W	
9.3	8	0	11	0	0	0	0	0	0	0	0	0	14
18.7	10	11	12	0	12	5	8	0	7	0	9	8	15
37.5	13	12	13	11	13	8	11	0	10	0	9	13	-
75	13	12	14	11	13	8	13	9	14	7	11	14	-
150	15	13	18	11	-	15	-	10	16	9	14	-	-

4W means one week after the first booster, and 5W means one week after the second booster. Figures indicated diameters of hemorrhage in mm.

Table 5. Antibody levels after the 3rd booster

Rabbits	7	11	18	20	3	2	9	10	Control	
Venom (γ)	(im)				(ic)					
									im	ic
0.25	-	-	-	-	-	-	-	-	0	0
0.5	-	-	-	-	-	-	-	-	1	0
1.1	-	-	-	-	-	-	-	-	1	8
2.3	-	-	-	-	-	-	-	-	1	9
4.6	0	0	0.5	0	-	-	-	-	1	11
9.3	0	0	1	0.5	0	0	4	0	1.5	12
18.7	0.5	1	1.5	1.5	11	12	12	0	2	13
37.5	1	1	2	1	13	13	15	13	3	-
75	3	3	3	2.5	17	14	16	15	3	-
150	3	3	3	3	17	15	22	-	3	-

Figures in im are mean score of local lesion of mice, and figures in ic are mean diameter of hemorrhage of rabbit represented in mm.

c. Mice weighing 20 gr were injected subcutaneously with 0.5 mg of toxoided venom of A. acutus in amount of 0.1 ml. There were no signs of hemorrhage or intoxication in rabbits which were immunized with the toxoided venom. Three weeks after the injection, two booster of 0.25 mg of toxoided venom were injected at the interval of two week. Then, 5 booster of 0.5 mg of toxoided venom were injected at the interval of one week. The total immunizing dose of toxoided venom was 3 mg. One week after the last booster, every five mice of seven groups were challenged with untreated venom of A. acutus ranging from 0.2 mg to 0.8 mg. In controls, the same amount of venoms were injected into the same numbers of mice. Twenty-four hours after the challenge, antilethal effect in mice which were immunized with toxoid were inspected.

As shown in table 6, more mice of immunized group than that of unimmunized one survived, although the data were not statistically.

Antibody level in blood of immunized mice showed that 0.1 ml of sera neutralized 9.3 γ of untreated venom of A. acutus, whereas the same amount of sera of unimmunized mice did not neutralize 1.1 γ of the venom (table 7.).

Table 6. Antilethal effects in mice immunized with toxoided venom of A. acutus

Venom (mg)	Immunized mice	Unimmunized mice
0.2	0/5	0/5
0.3	0/5	2/5
0.4	2/5	2/5
0.5	2/5	1/5
0.6	3/5	4/5
0.7	2/5	5/5
0.8	4/5	5/5

Numerator indicates the number of death. Denominators indicates the numbers of mice used.

Table 7. Antibody levels in blood of mice

Venoms (γ)	Immunized mice	Unimmunized mice
0.5	-	0
1.1	0	7
2.3	0	8
4.6	0	10
9.3	0	11
18.7	10	12
37.5	12	-

Antibody levels were measured by intracutaneous method in rabbits. Figures indicated diameters of hemorrhage in mm.

d. Antigenicity of Toxoided venom of T. mucrosquamatus:
Rabbits were injected subcutaneously with 2 mg of toxoided venom of T. mucrosquamatus which was treated by DHTA. Four weeks after, they were boostered four times with the same amount of toxoid at the interval of a week. One week of the last booster, some of immunized rabbits were challenged intramuscullarly with 2.4 mg or 4.8 mg of untreated venoms of T. mucrosquamatus, T. stejnegri and T. elegans.

Twenty-four after the challenge, those rabbits were sacrificed and inspected local lesions. Antibody levels of immunized rabbits were also tested.

As shown in Table 8, 9, and 10, local lesions both edema, hemorrhage and necrosis were much improved as compared with that in controls, although the protection of lesions were highest in the challenge of homologous venom, and least effective against the challenge of venom of T. stejnegri.

The same tendency was seen in antibody level as shown in Table 11 and 12. Thus, 0.1 ml of sera of immunized rabbits neutralized 9.3γ of venom (equivalent to 16 mhd) by the intracutaneous method in rabbit in homologous system. On the other hand, the same amount of sera of immunized rabbits neutralized 9.3 or 4.6γ of venom of T. elegans, and 4.6 or 0.5γ of venom of T. stejnegri.

e. Antigenicity of toxoided of venom of T. stejnegri:
As shown in table 13, 14 and 15, local lesions were much improved by challenges of both homologous and heterologous venoms as shown in previous experiments.

Neutralization tests of sera of immunized rabbits were shown in table 16 and 17. 0.1 ml of sera neutralized 4.6 or 2.3γ of venom of T. stejnegri and T. elegans, and 2.3 or 1.1γof venom of T. mucrosquamatus.

f. Antigenicity of Toxoid of venom of T. elegans:
As shown in table 18, 19 and 20, local lesions were much improved by the challenge of three kinds of venom as above.

Neutralization tests in table 21 and 22 showed that 0.1 ml of immunized sera neutralized 9.3 or 18.7γ (equivalent to 32 mhd) of venom of T. elegans, 4.6 or 2.3γ of venom of T. mucrosquamatus and less effective against venom of T. stejnegri.

Table 8. Antitoxic effects against venom of T. mucrosquamatus in rabbits immunized with toxoided venom of T. mucrosquamatus

Challenged Venom of	No. of Rabbits	Edema	Hemorrhage	Necrosis
T. Mucrosq.	1	(-)	+	(-)
	2	+	+	(-)
	3	+	+	(-)
2.4 mg	4 (control)	++	++	(-)
	5 (")	++	++	4 x 10 x 5
	6	+	+	5 x 5 x 5
	7	+	+	(-)
	8	+	++	10 x 10 x 8
4.8 mg	9 (control)	+++	++	30 x 11 x 12
	10 (")	+++	+++	30 x 10 x 8

Rabbits of no. 4 and 5, 9 and 10 were used as controls. Degree of lesions were indicated as follows: Swelling -- + slight edema with serous exudate, ++ marked swelling with hemorrhagic exudate, +++ strong swelling with hemorrhagic exudate. Hemorrhage -- + hemorrhage of pink or red and localized around the site of injection, ++ hemorrhage of red or darkred reached whole the thigh, +++ hemorrhage reached abdominal side. Size of necrosis is inspected by cutting muscles. Figure in necrosis indicated the size of lesion in mm.

Table 9. Antitoxic effects against venom of T. stejnegri in rabbits immunized with toxoided venom of T. mucrosquamatus

Challenged Venom of	No. of Rabbits	Edema	Hemorrhage	Necrosis
T. stejnegri	9	++	++	15 x 10 x 8
	10	++	+	10 x 5 x 3
2.4 mg	11	+++	++	(-)
	14 (control)	+++	+++	40 x 10 x 10
	15 (")	+++	++	15 x 5 x 3
	12	++	++	20 x 10 x 7
	13	++	+	(-)
4.8 mg	16 (control)	+++	+++	50 x 15 x 15
	17 (")	+++	+++	40 x 10 x 5

See foot note of table 1. Lesion of venom of T. stejnegri is characterized by the strong and bad smell.

Table 10. Antitoxic effects against venom of T. elegans in rabbits immunized with toxoided venom of T. mucrosquamatus

Challenged Venom of	No. of Rabbits	Edema	Hemorrhage	Necrosis
T. elegans	4	+	++	(-)
	14	+	+	(-)
2.4 mg	20 (control)	+++	++	(-)
	21 (control)	+++	+++	20 x 10 x 10
	15	++	++	(-)
	19	++	+	10 x 5 x 5
4.8 mg	22 (control)	+++	+++	20 x 10 x 5
	23 (control)	+++	+++	30 x 10 x 5

Lesions were characterized by grey-yellow-reddish color of necrosis. Edema were marked in controls.

Table 11. Antibody levels measured by intracutaneous method in rabbits against venom of T. mucrosquamatus.

Rabbits No. / Venom (γ)	1	5	8	11	16	Untreated
0.5	-	-	-	-	-	8 mm.
1.1	0	0	0	0	0	8
2.3	0	0	0	0	0	14
4.6	0	0	0	0	0	15
9.3	0	0	0	4	0	not tested
18.7	8	10	15	11	12	"

Test sera were taken from rabbits one week after the second booster. 0.1 ml of venom and serum were mixed and incubated at 37 degree C for one hour and then injected intracutaneously into rabbits. 24 hours after the injection, rabbits were sacrificed and the skin are taken off and sticked on the glass plate. Hemorrhage are observed reversing the glass plate, and the degree of lesions are measured by mm diameter.

Table 12. Antibody levels against venom of T. stejnegri

Rabbits No.	10	16	Control	14	19	Control
Venom (γ)	(Venom of T. stejnegri)			(Venom of T. elegans)		
0.25	-	-	-	-	-	-
0.5	-	-	9	-	-	9
1.1	10	0	10	0	0	11
2.3	10	0	10	0	0	11
4.6	11	0	10	0	0	12
9.3	11	5	11	0	8	14
18.7	14	12	12	11	8	not tested

See footnote of Table 4.

Table 13. Antitoxic effects against venom of T. stejnegri in rabbits immunized with toxoided venom of T. stejnegri

Challenged Venom of	No. of Rabbits	Edema	Hemorrhage	Necrosis
T. stejnegri	1	+	+	0
	3	0	+	0
1.2 mg	4	+	0	0
	12(control)	++	++	0
	13(")	++	+	0
	5	+	+	0
	6	++	+	35 x 15 x 10
	7	0	0	0
2.4 mg	14(control)	++	++	15 x 5 x 5
	15(")	+++	++	20 x 10 x 5
	9	++	+	0
	10	++	+	10 x 7 x 4
	11	++	++	0
4.8 mg	16(control)	+++	+++	45 x 20 x 12
	17(")	+++	+++	50 x 20 x 20

See footnote of Table 1.

Table 14. Antitoxic effects against venom of T. elegans in rabbits immunized with toxoided venom of T. stejnegri

Challenged Venom of	No. of Rabbits	Edema	Hemorrhage	Necrosis
T. elegans	15	0	+	0
	16	+	+	0
1.2 mg	21(control)	++	++	20 x 10 x 10
	22(")	++	+	0
	20	++	+	0
2.4 mg	18	++	++	0
	23(control)	+++	+++	35 x 15 x 8
	24(control)	++	++	35 x 10 x 5

Table 15. Antitoxic effects against venom of T. mucrosquamatus in rabbits immunized with toxoided venom of T. stejnegri

Challenged Venom of	No. of Rabbits	Edema	Hemorrhage	Necrosis
T. mucrosq.	8	+	+	0
	12	+	+	0
1.2 mg	25(control)	+	+	0
	26(control)	++	++	35 x 20 x 15
	13	+	+	10 x 5 x 5
	14	++	++	0
2.4 mg	27(control)	+++	+	0
	28(")	+++	++	45 x 20 x 10

Table 16. Antibody levels of rabbits immunized with toxoided venom of T. stejnegri against venom of T. stejnegri

Rabbits No. / Venom (γ)	2	4	6	8	9	11	Control
T. stejnegri							
0.25	-	-	-	-	-	-	0.5
0.5	-	-	-	-	-	-	1.0
1.1	0	0	0	0	0	0	1.0
2.3	0.5	0	0	1.0	0	0.5	1.5
4.6	1.0	0	1.0	1.0	1.0	1.0	3.0
9.3	1.0	1.0	1.0	1.0	1.0	2.0	3.0
18.7	1.5	3.0	2.5	2.5	2.5	3.0	3.0
37.5	2.3	3.0	3.0	3.0	3.0	3.0	-

Antibody level of sera was investigated intramuscular method in mice. 0.1 ml of rabbit serum or pooled sera of mice and 0.1 ml of ranging dose of venom are mixed and incubated at 37 degree C for one hour, and then injected intramuscularly into the leg of mice. 24 hours after the injection, the local lesions and death or survival are observed. Mice are sacrificed and skin of the legs are cut off and the presence or absence of hemorrhage is observed. Degree of local lesion are indicated as follows: 0 -- no lesion or hemorrhage at the point of injection 1 -- hemorrhage in one third of the thigh. 2 -- hemorrhage in two third of the thigh. 3 -- hemorrhage reach the trunk. And then, calculated the mean score of lesions of mice tested.

Table 17. Antibody levels of rabbits immunized with toxoided venom of T. stejnegriagainst venom of T. elegans

Rabbits No.	15	20	18	Control	12	14	Control
Venom (γ)	(Venom of T. elegans)				(Venom of T. mucrosqu.)		
0.125	-	-	-	-	-	-	-
0.25	-	-	-	8	-	-	-
0.5	-	-	-	12	-	-	6
1.1	0	0	0	12	0	0	8
2.3	0	0	12	13	0	7	11
4.6	0	7	15	15	7	9	13
9.3	3	8	16	18	9	11	14
18.7	8	8	19	-	12	15	-
37.5	9	8	-	-	-	15	-

See footnote of Table 4.

Table 18. Antitoxic effects against venom of T. elegans in rabbits immunized with toxoided venom of T. elegans.

Challenged Venom of	No. of Rabbits	Edema	Hemorrhage	Necrosis
T. elegans	1	+	+	0
	3	0	+	0
	4	0	+	0
1.2 mg				
	20(control)	+	+	20 x 10 x 4
	21(")	+	+	0
	6	+	+	0
	7	+	+	0
	8	++	+	15 x 10 x 8
2.4 mg				
	22(control)	++	++	10 x 8 x 5
	23(")	++	++	20 x 10 x 5
	9	++	+	0
	10	+	+	0
	11	++	+	15 x 10 x 8
4.8 mg				
	24(control)	+++	+++	20 x 15 x 8
	25(")	+++	+++	35 x 18 x 13

See footnote of Table 1.

Table 19. Antitoxic effects against venom of T. mucrosquamatus in rabbits immunized with toxoided venom of T. elegans

Challenged Venom of	No. of Rabbits	Edema	Hemorrhage	Necrosis
T. mucrosq.	16	+	+	0
	17	+	+	0
1.2 mg				
	26(control)	++	++	20 x 13 x 9
	27(")	++	++	0
	19	++	+	0
	20	+++	++	10 x 5 x 3
2.4 mg				
	28(control)	+++	++	45 x 10 x 5
	29(")	+++	++	30 x 10 x 5

See footnote of Table 1.

Table 20. Antitoxic effects against venom of T. stejnegri in rabbits immunized with toxoided venom of T. elegans.

Challenged Venom of	No. of Rabbits	Edema	Hemorrhage	Necrosis
T. stejnegri	14	+++	+	0
	15	++	++	0
1.2 mg				
	26(control)	++	++	0
	27(")	+++	++	20 x 15 x 8
	12	+++	++	0
	13	+	+	0
2.4 mg				
	28(control)	++	++	10 x 8 x 5
	29(control)	+++	++	30 x 10 x 7

See footnote of Table 1.

Table 21. Antibody levels of rabbits immunized with toxoided venom of T. elegans against venom of T. elegans

Rabbits No. / Venom(γ)	4	8	9	13	18	19	Control
T. elegans							
0.25	-	-	-	-	-	-	0
0.5	-	-	-	-	-	-	1.0
1.1	0	0	0	0	0	0	1.0
2.3	0	0	0	0	0	0	1.0
4.6	0	0	0	1.0	1.0	0	1.5
9.3	0	0	0	1.0	1.0	0	2.0
18.7	1.5	0	0	2.0	2.0	0.5	3.0
37.5	2.0	1.5	1.0	3.0	2.5	1.5	3.0

See footnote of Table 9.

Table 22. Antibody levels of rabbits immunized with toxoided venom o of T. elegans against venoms of T. mucrosquamatus and T. stejnegri

Rabbits No. / Venom(γ)	16 (Venom of T. mucrosquamatus)	19	5	Control	12 (Venom of T. stejnegri.)	14	Contr.
0.25	-	-	-	4	-	-	8
0.5	-	-	-	4	-	-	8
1.1	0	0	0	9	12	9	9
2.3	0	0	0	11	12	11	11
4.6	6	6	0	12	12	11	13
9.3	8	?	0	12	12	11	13
18.7	9	?	8	-	13	-	-
37.5	9	?	8	-	-	-	-

See footnote of Table 4.

9. Antigenicity of polyvalent toxoided venom of T. mucrosquamatus, T. stejnegri and Agkistrodon acutus. Rabbits were injected subcutaneously with 3 mg of toxoid which is consist of the same amount of three kinds of venom shown above in amount of 0.3 ml as a dose. Three weeks after, they were boostered two of three times with 0.5 mg of toxoid and the last with 3 mg of toxoid. One week after the last booster, some of immunized rabbits were challenged intramuscularly with 1.2, 2.4 and 4.8 mg of three kinds of untreated venom.

As shown in table 16, 17 and 18, local lesions due to the challenge of three kinds of venom were much improved.

Neutralization tests in table 19 and 20 shows that 0.1 ml of sera of rabbits which has been received two booster shots neutralized 2.3 γ (4mhd) of venom of T. stejnegri and T. mucrosquamatus and 9.3 γ (8mhd) of venom of A. acutus. Neutralizing effects of sera of rabbits which received one more booster increased so that the same amount of sera neutralized 9.3γ (rabbit No. 23) of venom of T. stejnegri and 4.6γ of T. mucrosquamatus and 37.5γ (rabbit No. 1) of venom of A. acutus.

Thus, it is suggested that polyvalent toxoid is good antigenic against local hemorrhagic effects of venom of Taiwanese vipers.

10. Venom of Bungarus multicinctus: The venom was added with the same amount of DHTA and incubated at 37 degree C for one hour. 0.05 mg of the toxoided venom were injected subcutaneously into mice in amount of 0.1 ml. The mice showed any sign of intxication by the injection of toxoid. Three weeks after the injection, 0.05 mg, 0.1 mg 0.1 mg and 0.2 mg of toxoided venom were boostered at the interval of a week. One week after the 3rd and the last booster, mice were challenged intramuscularly with varying dose of venom of B. multicinctus ranging from 2.3γ to 75γ. The results are indicated in table 27. Both group of immunized mice protected from the challenged venom of 9.3γ, and another group of immunized mice which received 1.7γ of venom died in 24 hours after the challenge of venom, whereas untreated mice died one hour after the challenge. Minimum lethal dose of venom was 4.6γ.

LD50 of the venom against both immunized and unimmunized mice were determined in another tests. As shown in table 28, LD50 of the venom in immunized mice was 13.5γ, whereas 2.7γ was that of unimmunized mice.

Table 23. Antitoxic effects against venom of T. stejnegri in rabbits immunized with polivalent toxoid of venoms of T. stejnegri, T. mucrosquamatus and A. acutus

Challenged Venom of	No. of Rabbits	Edema	Hemorrhage	Necrosis
T. stejnegri	13	++	+	(-)
	23	++	+	(-)
	24	+	+	(-)
1.2 mg				
	43(control)	++	++	15 x 10 x 5
	44(")	+++	++	10 x 10 x 8
	25	+++	++	(-)
	26	+++	++	(-)
	27	+++	+++	(-)
2.4 mg				
	45(control)	+++	+++	32 x 14 x 10
	46(")	+++	++++	35 x 17 x 10
	21	+++	+++	(-)
	28	++	++	(-)
	29	+++	+++	(-)
4.8 mg				
	47(control)	+++	++++	35 x 15 x 13
	48(")	+++	++++	35 x 13 x 10

Table 24. Antitoxic effects against venom of T. mucrosquamatus in rabbits immunized with polyvalent toxoid of venoms of T. stejnegri, T. mucrosquamatus and A. acutus.

Challenged Venom of	No. of Rabbits	Edema	Hemorrhage	Necrosis
T. mucrosquamatus	12	+	+	(-)
	15	+	+	(-)
	14	+	+	20 x 7 x 5
1.2 mg				
	37(control)	+	+	(-)
	38(")	+	+	25 x 12 x 9
	17	++	+	19 x 8 x 10
	18	++	+	(-)
	19	++	+	(-)
2.4 mg				
	39(control)	++	++	35 x 20 x 15
	40(")	+++	++	17 x 10 x 10
	16	+++	++	20 x 10 x 10
	19	++++	++	(-)
	20	+++	++	(-)
4.8 mg				
	41(control)	+++	++++	28 x 13 x 10
	42(")	+++	++	24 x 15 x 12

Table 25. Antitoxic effects against venom of A. acutus in rabbits immunized with polyvalent toxoid of venoms of T. stejnegri, T. mucrosquamatus and A. acutus

Callenged Venom of	No. of Rabbits	Edema	Hemorrhage	Necrosis
A. acutus	1	+	+	(-)
	2	+	+	(-)
	3	+	+	(-)
1.2 mg				
	31(control)	+	+	20 x 9 x 7
	32(")	+	+	24 x 10 x 8
	4	+	+	(-)
	5	+	+	(-)
	6	+	+	(-)
2.4 mg				
	33(control)	+	+	15 x 9 x 6
	34(")	+	+	(-)
	7	++	+	(-)
	8	++	+	(-)
4.8 mg	11	++	+	(-)
	35(control)	++	++	25 x 10 x 14
	36(")	++	++	35 x 12 x 14

Table 26. Antibody levels measured by intramuscular method in rabbits which were immunized with polyvalent toxoid of venoms of T. stejnegri, T. mucrosquamatus, and A. acutus against their venoms.

Rab. No. / Venom	Trimeresurus stejnegri			T. mucro-quamatus			Agkistrodon acutus		
	13	20	49	12	16	50	14	15	51
0.14	-	-	0	-	-	0.5	-	-	-
0.29	-	-	0.5	-	-	1.0	-	-	-
0.58	-	-	1.0	-	-	1.0	-	-	0.5
1.1	-	-	1.0	-	-	1.0	-	-	1.0
2.3	0.5	1.0	1.0	0.5	0	1.5	0	0	1.0
4.6	1.5	1.5	2.5	1.0	1.0	2.0	0	0	1.0
9.3	1.5	2.0	3.0	1.0	1.0	2.0	0	0	1.0
18.7	2.5	3.0	3.0	1.5	1.5	3.0	1.0	1.0	1.5
37.5	3.0	3.0	-	3.0	3.0	3.0	1.5	1.5	3.0

Sera were taken from rabbits which received two boosters.

Table 27. Antitoxic effects of immunized mice against venom of B. multicinctus

Mice Venom (γ)	* Immunized	Unimmunized	** Immunized	Unimmunized
2.3	0/4	0/4	0/5	0/5
4.6	0/4	1/4	0/5	5/5
9.3	0/4	4/4	0/5	5/5
18.7	4/4***	4/4	5/5***	5/5
37.5	4/4	-	5/5	5/5
75	4/4	-	5/5	-

*Mice were challenged after the third booster. **Mice were challenged after the last booster. ***Death of mice prolonged 24 hours. Numerator indicates the number of death. Denominators indicates the numbers of mice used.

Table 28. LD 50 of venom of B. multicinctus for immunized and unimmunized mice

Venom (γ)	Mice Immunized	Venom (γ)	Mice Unimmunized
9	0/5	2	0/5
10	0/5	4	3/5
12	2/5	5	5/5
14	2/5	6	5/5
16	4/5	-	-
18	5/5	-	-
13.5γ (LD50)		2.72γ (LD50)	

3. Analysis of results of the current immunization program against habu venom now being conducted in the Amami and Ryukyu Islands:

As shown in table 28, 43, 446 volunteers in the Amami and the Ryukyu Islands received habu venom which is treated by dihydrothioctic acid (DHTA) in a period from 1965 to 1967.

0.5 ml of toxoided venom which is consist of 2.5 mg of venom and the same amount of DHTA and the mixture is incubated at 37 degree C for one hour and freeze-dried is injected subcutaneously as one dose. Three or four weeks and then half or one year after booster shots are given.

Side reactions by injection of toxoided venom is investigated for 5,761 persons. These include localized pain and swelling which persisted as long as a week. Generalized sysptoms such as fever were few. Neutralizing titer of the sera of immunized persons were not so high as that of habu antivenin used to treatment.

However, 0.1 ml of sera from persons who had received the toxoid neutralized from one to sixteen minimum hemorrhagic dose (mhd) of untreated venom.

Clinical records of 143 habu-bite patients as shown in table 29 who had previously received one or more injections indicated that only three individuals showed slight motor disturbances in which motor disturbance and deformity caused in one of the three patients is not due to habu-venom but to the intensified touniquet after the bite. On the other hand, of 1,567 patients who had not participated in the immunization program, 118 patients suffered from severe necrosis in which 18 persons accompanied by severe generalized symptoms and 80 persons left motor disturbances after the recovery of wound.

As the results reported above, it is suggested that the toxoided venom is effective against local legion caused by the venom. However, lethal cases occured on two persons who received shots previously, although 19 persons who received no shots died from habu bite.

Table 29. Number of persons who received toxoid

Frequency / Areas	1	2	3	4	5	Total
Amami Is.	14,117 (39)	14,588 (48)	10,623 (34)	856 (7)	50 (1)	40,234 (129)
Okinawa	527 (2)	615 (4)	590 (5)	775 (3)	705 (0)	3,212 (14)
Total	14,644 (41)	15,203 (52)	11,213 (39)	1,631 (10)	755 (1)	43,446 (143)

Number in paremtheses indicate that of habu bite.

Table 30. Relationship between immunization and prognosis of habu-bite

Amami Is.	No. Patients	No. Inspected	Necrosis	Death
1965	247	232: T 5	1(1)	0
		O 227	22(18)	2
1966	284	265: T 35	1(1)	0
		O 230	27(20)	1
1967	286	283: T 89	2(0)	2
		O 194	23(14)	2
Subtotal	817	780: T 129	4(2)	2
		O 651	75(56)	5
Ryukyu Is.				
1965	367	321: T 0	0	0
		O 321	18(6)	7
1966	350	347: T 9	0	0
		O 338	11(7)	2
1967	373	262: T 5	1(1)	0
		O 257	14(11)	5
Subtotal	1,090	930: T 14	1(1)	0
		O 916	43(24)	14
Total	1,907	1,710: T 143	5(3)	2
		O 1567	118(80)	19

Number in parentheses indicate patients who left motor disturbance after the recovery of wounds. T: patients who received toxoid. O: patient who did not receive toxoid.

Discussion

There are some difficulty to collect Taiwanese snake venoms. The one is that yields of venom of B. multicinctus and T. stejnegri are usually so small that so many snakes should be collected to obtain venom available to study of toxoid. The other is that the venom is not controlled by the government but is managed only commercially in Taiwan. Therefore, milking from snakes should be done by the reseacher himself to avoid error in identification of venom.

Inactivation of venoms of A. acutus, T. mucrosquamatus, T. stejnegri, T. elegans and B. multicinctus was easily achieved by the same dose of DHTA as already reported.

It is suggested that there are close immunological similarity amorg venoms of T. mucrosquamatus, T. stejnegri and T. elegans as well as the geographycal distributions of them is close. It is also sugeted that the antigenicity of polyvalent toxoid is improved by the cooperation of the venoms included in the toxoid, in spite of the amount of the venoms are more smaller than that contained in momovalent toxoid.

Both toxoid of venom of Naja n. atra and polyvalent toxoid of neurotoxic venoms are now under investigation.

It has been found that analysis of field trial is not so easy because there are so many factors which influence to snake-bite such as method of treatment, time required to the treatment, site of bite, or amount of venom which introduced into the body of victims etc. Moreover, severe cases are less than ten per cent of all the snake-bites. In spite of those facts, results of survey suggested that snake venom toxoid is hopeful to decrease severe cases.

Conclusion

1. Five species of Taiwan se snake venom were collected in Taipei.

2. Rabbits and mice which were injected with toxoided venom of A. acutus treated by dihydrothioctic acid showed anti-hemorrhagic and anti-lethal effect against the venom. Neutralizing antibody against the venom also proved to increase as the immunization is repeated.

Mice which were immunized with venom of B. multicinctus treated by DHTA showed good anti-lethal effect.

Antigenicity of T. mucrosquamatus, T. stejnegri and T. elegans seems to be closely related, and rabbits which were immunized with one of the three kinds of venom showed cross-protection against local effect with each other.

Polyvalent toxoid of T. mucrosquamatus, T. stejnegri and A. acutus is good antigenic against local effect of venom of each other.

3. Those venoms which were treated by DHTA showed no signs of hemorrhage or intoxication.

4. An analysis of field trial of current immunization program suggested that toxoid is useful to decrease severe necrosis in habu-bites.

References

1. Sawai, Y., Makino, M., Miyasaki, S., Kato, K., Adachi, H., Mitsuhashi, S. and Okonogi, T.: Studies on the improvement of treatment of habu snake bite. 1. Studies on the improvement of habu snake antivenin. Japan. J. Exp. Med. 31, 137-150, 1961.

2. Sawai, Y., Makino, M., Miyasaki, S., Kawamura, Y., Mitsuhashi, S. and Okonogi, T.: Studies on the improvement of treatment of habu snake (Trimeresurus flavoviridis) bite. 3. Clinical analysis and medical treatment of habu snake bite. Japan. J. Exp. Med., 32, 117-138, 1962.

3. Sawai, Y., Makino, M. and Kawamura, Y.: Studies on the antitoxic action of dihydrolipoic acid (dihydrothioctic acid) and tetracycline against habu snake (Trimeresurus flavoviridis) venom. Venomous and Poisonous Animals and Noxious Plants of the Pacific Area, 327-335, 1963, Pergamon Press.

4. Sawai, Y., Kawamura, Y., Makino, M., Fukuyama, T., Lin, Y. H., Kuribayashi, H. and Ishii, T.: Studies on the habu snake venom toxoid. 1. The antigenicity of habu venom toxoid. Japan. J. Bact., 21, 32-41, 1966 (in Japanese--English abstract).

5. Sawai, Y., Kawamura, Y., Fukuyama, T., Kawai, J., Shimizu, T., Lin, Y. H., Ebisawa, I., Okonogi, T., Honma, M. and Shimozono, C.: Studies on the prophylaxis against habu snake bite by snake venom toxoid. 1. On the side reaction and antibody level after the immunization of the toxoid. Japan. J. Bact., 21, 88-91, 1966 (in Japanese, English abstract).

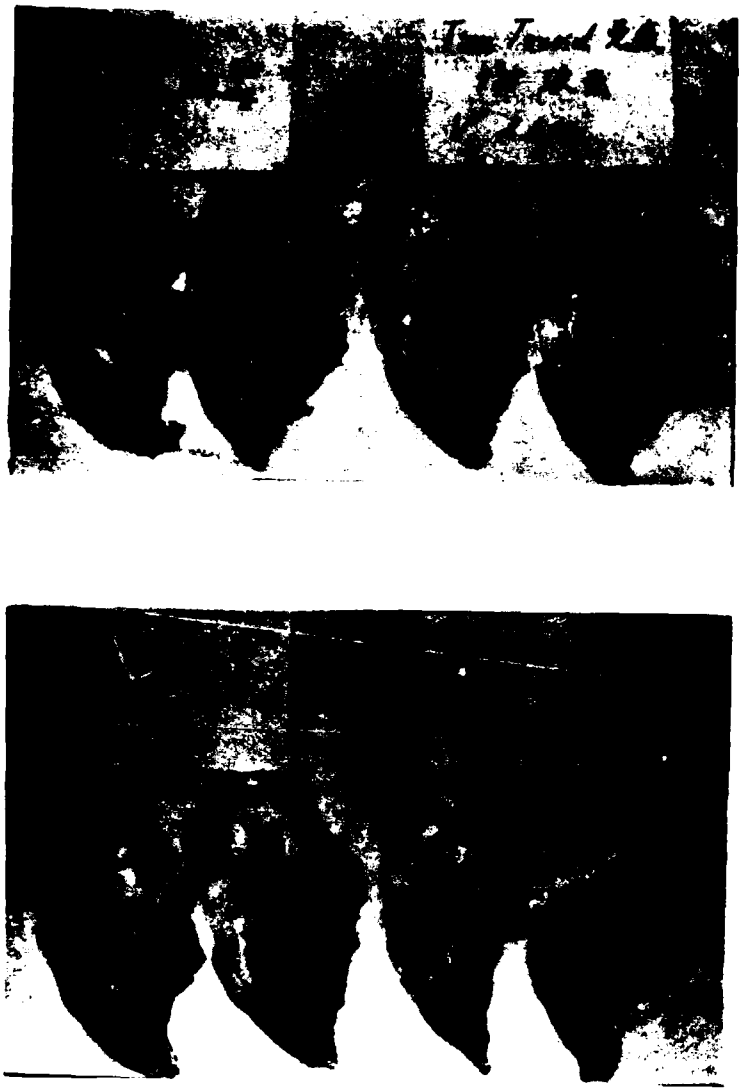
6. Miyagi, F., Hokama, Z., Yamakawa, M., Miyagi, E., Onishi, K., Uechi, S., Chibana, K., Sawai, Y., Kawamura, Y., Fukuyama, T., Oshiro, S., Ikemiya, S. and Yaka, I: Studies on the prophylaxis against habu snake (Trimeresurus flavoviridis) bite by snake venom toxoid. 2. On the side reaction of toxoid of venom Okinawa. Japan. J. Bact., 21, 206-209, 1966 (in Japanese, English abstract).

7. Sawai, Y., Kawamura, Y., Fukuyama, T., Shimizu, T., Okonogi,

T., Hattori, Z. and Igarashi, I.: Studies on the habu snake toxoid. 2. Studies on the side reaction in mice. Japan. J. Bact., 22, 67-72, 1967 (in Japanese, English abstruct).

8. Sawai, Y., Kawamura, Y., Ebihara, I., Okonogi, T., Hokama, Z. and Yamakawa, M.: Studies on the improvement of treatment of habu-bites.6, Snake-bites on Amami and Ryukyu Islands in 1964. Japan. J. Exp. Med., 37, 51-59, 1967.

9. Sawai, Y., Kawamura, Y., Fukuyama, T. and Keegan, H. L.: Studies on the inactivation of snake venom by dihydrothioctic acid. Japan. J. Exp. Med., 37, 121-128, 1967.



Upper: Protection of local lesions in rabbits (two on the right) which were immunized with toxoid of venom of T. mucrosquamatus, and challenged with 2.4 mg of the same venom. In controls (two on the left), severe necrosis and hemorrhage occurred.
Bottom. The same as above. The amount of venom challenged is 4.8 mg.

D-I-S-T-R-I-B-U-T-I-O-N

The distribution of this report as made by USA R&D Gp (FE) is as follows:

Director of Army Research (3)
Office of the Chief Research and Development, DA
ATTN: Chief, Research Programs Office
Washington, D. C. 20310

Army Attache, American Embassy, Tokyo, Japan (1)

U. S. Army Medical Research and Development Command (4)
Washington, D. C. 20315

Defense Documentation Center, Cameron Station (20)
Alexandria, Virginia. ATTN: TISIA-2

Office of Primary Scientific Liaison (2)
Preventive Medicine Research Branch
U.S. Army Medical Research and Development Command
Washington, D. C. 20315

Office of Scientific Cognizance (1)
Colonel Hugh L. Keegan
U.S. Army Element
Defense Research Office, Latin America
American Embassy
APO New York 09676

Unclassified
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) Laboratory of Snake Venom, Snake Center Gunma Prefecture, Japan	2a. REPORT SECURITY CLASSIFICATION Unclassified 2b. GROUP

3. REPORT TITLE

A STUDY ON THE PROPHYLACTIC TOXID AGAINST VENOMS OF CERTAIN ASIAN SNAKES (U)

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Final Report No.2, 30 June 1967 - 29 June 1968

5. AUTHOR(S) (First name, middle initial, last name)

Yoshio Sawai

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
July 1968	32	9

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
DA-JB17-67-C-0610 b. PROJECT NO. 3A014501B71P c. Task 10 102FE d.	FE-356-2 9b. OTHER REPORT NOS (Any other numbers that may be assigned this report)

10. DISTRIBUTION STATEMENT

This document has been approved for public release and sale; its distribution is unlimited.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	U.S. Army R&D Group (Far East) APO San Francisco 96343

13. ABSTRACT

The purpose of this study is to help the medical treatment of severe poisonous snake-bite, because there would be a limit in serum treatment of patient who received a large amount of venom. To solute this problem, the writer accumulated data on prophylactic immunization against habu snake venom which is dommon in South West Islands of Japan. This report concerns an attempt to immunize experimental animals with Taiwanese snake venoms which were inactivated by dihydrothioctic acid. Reports on surveys of field trial of prophylactic shots with toxoided venom in habu-infested area is also added here. Five kinds of venom were collected, milked and freezedried from snakes at Taipei of Taiwan. At first, two kinds of Agkistrodon acutus and Bungarus multicinctus were inactivated by dihydrothioctic acid and injected into rabbits and mice. Those immunized animals were challenged intramuscularly by each kind of untreated venom for the test of antigenicity. Blood levels of antibody of immunized animals were also investigated. The results showed that hemorrhagic effects of venom of A. acutus were prevented in considerable degree in immunized rabbits, although antibody of sera of immunized animals were not so high as antivenin for treatment. Antilethal activity of mice which were treated by venom toxoid of B. multicinctus increased definitely. In the next, immunological relationship of venom of T. mucrosquamatus, T. stejnegri and T. elegans were investigated. Rabbits were immunized with each of those venom toxoid, and then crossly challenged by the those venom respectively. The results indicated that those venoms were immunologically correlated with each other. Finally, polyvalent toxoid of venom of T. mucrosquamatus, T. stejnegri and A. acutus was also proved to be good antigenic in rabbits by the same method. Analysis of results of current immunization program from 1965 to 1967 suggested that victims who received toxoided venom previously recovered without severe local lesion. (Author)

DD FORM 1473

Unclassified
Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Snakes						
Venom						
Antivenin						
Toxoid						
Purification						
Immunization						
Japan						