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U.S. ARMY MEDICAL RESEARCH LABORATORY

PORT KNOX, KENTUCKY

REPORT NO. 594

ACTIVE IMMUNIZATION OF A HUMAN AGAINST
NAJA NAJA VENOM

A Preliminary Report

Capt Herschel H. Flowers, VC

CATALOGUED BY DDC
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UNITED STATES ARMY
MEDICAL RESEARCH AND DEVELOPMENT COMMAND 12 November 1963

NO OTS

Report Submitted 5 September 1963

Author

**Capt Herschel H. Flowers, VC
(DVM)**

Pathology Division

**The animals used in this study were handled in accordance with the
"Principles of Laboratory Animal Care" established by the National
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**ACTIVE IMMUNIZATION OF A HUMAN AGAINST
NAJA NAJA VENOM**

A Preliminary Report

Capt Herschel H. Flowers, VC

**Pathology Division
US ARMY MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky**

11 November 1963

**In-House Laboratory Initiated Research and Development
DA Project No. 3A013001A814**

USAMRL Report No. 594
DA Project No. 3A013001A814

ABSTRACT

**ACTIVE IMMUNIZATION OF A HUMAN AGAINST
NAJA NAJA VENOM**

A Preliminary Report

OBJECT

To determine whether or not a human subject could be immunized against the bite of the Indian cobra (Naja naja). To study the development and titer of neutralizing antibodies produced. To determine any pathological conditions produced by the injections during the course of immunization.

RESULTS

Tests demonstrated the theoretical neutralization potential of the subject's circulating globulin to be approximately 100 mg Naja naja venom. Examination of the subject during and after the course of the immunization schedule revealed development of no pathological conditions.

CONCLUSIONS

Using venoms with little or no local toxicity, it appears that man may be safely immunized.

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Colonel, VC
Director, Pathology Division

APPROVED: *Sven A. Bach*
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Director

ACTIVE IMMUNIZATION OF A HUMAN AGAINST NAJA NAJA VENOM

A Preliminary Report

Because of the complexity of venom proteins and their great local and systemic effects, active immunization has been confined to animals utilized in the production of commercial antivenins. In this study the feasibility of a safe and practical method of immunization of man against snakebite has been explored.

Parrish and Pollard (1) found that immunity against the effect of snakebite did not exist among 14 human subjects, each of whom had been bitten two or more times by Crotalid snakes. However, the development of a persistent immunity might not be anticipated in this group because envenomation occurred from 2-year to 14-year intervals, and the members of the group were also treated with antivenins following snakebite. The first observation of active immunization against snakebite was reported by Haast and Winer (2). A subject (human) was given repeated injections of crude cobra venom over a period of 2-1/2 years. A maximal injection of 40 mg of venom was tolerated, and this quantity of venom is lethal in the absence of immunity. Immunological tests were not performed with the serum or globulin to determine the degree of protection afforded by these injections, but the 40 mg immunizing dose of venom he tolerated is significant.

Wiener (3) described in one human subject the titer rise and persistence effected by repeated injections of Australian Tiger Snake venom. This subject survived the bite of a snake of this species with little difficulty subsequent to his immunization.

In this laboratory, in an attempt to measure the development of neutralizing antibodies, a human subject was given 17 injections containing increasing amounts of Indian cobra (Naja naja) venom. Venom solutions were passed through a millipore filter and then added to an equal volume of a 4% Sodium Alginic adjuvant.* The 17 injections were given over a period of 5 months (Fig. 1). Ten ml of blood was withdrawn from the subject after the 4th, 7th, and 10th injections. Five hundred ml of blood was withdrawn after the 11th injection, and

*Sodium Alinate Adjuvant, Colab Laboratories, Box 66, Chicago Heights, Illinois.

1 liter after the 17th. Globulins were separated from serum by ammonium sulfate precipitation, and the antivenin titer was determined by neutralization of Naja naja venom in 18-20 Gm Swiss mice. Identical neutralization tests were conducted by a commercial laboratory (Fig. 2).

The results of this study show a development of active immunization against cobra venom. One ml of the globulin solution neutralized 36 mouse LD₅₀s (.244 mg venom). Since it required 100 ml of whole blood to produce 1 Gm of the crude globulin, it is estimated on a blood volume basis that 40 Gm are present in the subject's circulation. This circulating globulin alone therefore should neutralize a dose of 100 mg cobra venom. This calculation does not take into account the extravascular globulins present in the area of envenomation, which themselves are capable of venom neutralization.

The subject receiving cobra venom was under observation by a physician. Periodic blood and blood chemistry examinations were conducted to determine possible pathological conditions arising from the injections. Examination of the subject during and after the injections indicated the absence of pathological response to the venom except for local swelling and pain which persisted from 1 to 3 days in the area of injection. For a 2-week period after the 16th injection of venom, the subject's blood pressure was elevated slightly.

The potential advantages of active immunization of man against envenomation have been recognized for many years. The presence of locally necrotizing enzymes in some venoms still presents a formidable obstacle. However, in venoms with little or no local toxicity it appears that man may be safely immunized.

ACKNOWLEDGEMENT

The author gratefully acknowledges Wyeth Laboratories, Marietta, Pennsylvania, for their assistance in neutralization tests described in this report.

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2. Haast, William E. and Melvin L. Winer. Complete and spontaneous recovery from a bite of a blue krait snake (Bungarus coeruleus). Amer. J. Trop. Med. and Hyg. 4: 1135, 1955.
3. Wiener, Saul. Active immunization of man against the venom of the Australian Tiger Snake (Notechis scutatus). Med. J. Australia, 1: 658, 1961.

IMMUNIZATION SCHEDULE

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100

Fig. 1. Immunization schedule of human subject.

DEVELOPMENT LAB

DEVELOPMENT REQUEST

Dr. Strode
Dr. Freedman
Mr. Lammert

Reference No. P-393

Trial Lot No. 537267

Project No. A-2111

Date April 2, 1963

To Dr. Criley From John Koenigsdorff
Subject Cobra Venom (Naja Naja - Capt. Flowers) vs.
Human Globulin (Capt. Flowers - dried.)
Issue August 22/53 Standard
Complaint No.

Human globulin restored to fluidity 1 cc. plus 0.2cc. diluent for
antivenin 537267.

Results

LD_{50} 160/mg
(1 LD_{50} = 0.3595 mg.)

Neutralization Globulin v.s. venom

1 cc. of human globulin solution as prepared
above neutralized 30 μ g. cobra venom
(0.251 = 0.0016/mg./cc.)
Test = 12.5 ml.

S.L. rep. 1

John Koenigsdorff

JK/srs.

Fig. 2. Results of neutralization tests of subject's globulin against Naja naja venom.

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