# THERAPEUTIC EXPERIMENTS WITH ANTICROTALUS AND ANTIMOCCASIN SERA.

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The thesis which forms the basis of this paper is the specificity of snake venoms and the therapeutic value of anticrotalus and antimoccasin snake sera. The subject in general is not a new one and has been dealt with in some of the aspects here considered by Calmette,[1] Kanthack,[2] C. J. Martin,[3] Lamb,[4] and Flexner and Noguchi.[5] Calmette's view that cobra antivenin is active against all kinds of snake venom and even the poison of scorpions has been shown to be erroneous, chiefly by the other investigators mentioned. The great increase in our knowledge of the physiological constitution of venom should make easily explicable the absence of interaction between the antisera of the venom of the Colubridæ and the Viperidæ, but up to the present time all the tests have been made with Calmette's antivenin, which is prepared with cobra venom chiefly. My previous studies with Crotalus and Ancistrodon venoms led me to prepare the respective antisera, and to use them in testing first the question of their specificity, and next the possibility of their employment

* This study was conducted while I was Research Assistant of the Carnegie Institution, Washington, D. C.

[1] Calmette.—Immunisation artificielle des animaux contre le venin des serpents. *La Semaine med.*, 1894, 76.

See also *Ann. de l'Inst. Pasteur*, 1894, vii, 275.

[2] Kanthack.—Rep. of Medical Officer of Loc. Gov. Board, London, 1895–1896, 235.

[3] C. J. Martin.—*Inter-colonial Medical Journal of Australia*, 1897, ii, 537.

Calmette, Ibid., 1898, iii, 192.

[4] Lamb.—Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India, New Series, Nos. 5 (1903), 10 (1904), 16 (1905).

[5] Flexner and Noguchi.—Upon the Production and Properties of Anticrotalus venin. *Journal of Med. Research*, 1904, New Series VI, 363.

as therapeutic agents. The wide prevalence of the rattlesnake and moccasin in the United States may, at some time, make it worth while to produce commercially antisera for their venoms, once it is proved that they are effective therapeutic agents. My experiments were made with anticrotalus and antimoccasin venins made in goats, and anticobra venin kindly supplied by Professor Calmette. The two main effects investigated were (1) the antihæmolytic and (2) the antitoxic.

In Table I the value of the several antisera in direct and cross neutralizing power for several venoms is shown. In A the antitoxic and in B the antihæmolytic values are given. Guinea-pigs were used for the experiments on toxicity, and defibrinated dog blood, in 2.5 per cent. suspension in 0.9 per cent. salt solution, for the tests on hæmolysis. In making the latter tests the blood suspensions, venom, and antivenin were kept at 37° C. for two hours and at room temperature for six hours, after which the readings were made colorimetrically.

The two sets of experiments given show the highly specific antitoxic and antihæmolytic action of the serum. The action is, however, not absolutely specific.

With this knowledge as a basis of further experimentation I proceeded to test the therapeutic value of the antisera. The method was to inject a number of guinea-pigs intraperitoneally with 2 m. l. d. of the venoms and one, two, three, and four hours later to inject the antisera, also intraperitoneally. The control pigs died in from three and a half to four and a half hours after receiving the poison.

Tables II and III give in summarized form the results upon which are based the conclusions that these antisera possess therapeutic properties of high value. Animals which show marked symptoms of venom poisoning can be rescued from certain death provided they are yet able to stand. When they once lie prostrate upon the bottom of the cage they cannot be saved; but I have repeatedly noticed that pigs do not survive this latter condition longer than fifteen to twenty minutes, and hence they are really moribund. In cases in which recovery takes place the improvement proceeds rapidly; the animals are well,

apparently, at the end of twenty-four hours, although the complete restoration of weight may require several days longer. The successfully treated animals remain well for an indefinite period.

The manner of determining the toxicity of Crotalus venom is to be learned by referring to a paper by Madsen and Noguchi, to be published in a subsequent number of this Journal. It may be stated, however, that one cubic centimeter of the anticrotalus serum neutralized *in vitro* 0.0025 gram of the corresponding venom or 2.5 times the quantity used in the therapeutic experiments. One cubic centimeter of the antimoccasin serum neutralized *in vitro* 0.002 gram of the venom (Table IV).

Calmette [6] and Fraser [7] had already made similar experiments with cobra antivenin, and my conclusions are similar to theirs. They state that if a sufficient dose of the antivenin be given within one and a half hours following the injection of a quantity of the cobra venom sufficing to cause death in controls in three to four hours, the life of the poisoned animals may be saved. As the time interval between administrations of venom and antivenin increased successful treatment became more uncertain. These experimenters also found that the length of the time interval could in part be overcome by adjusting the dose of the antivenin. The greater the interval between the poisoning and the antitoxin treatment, the larger the quantity of the antivenin required to rescue the animal, until a point in the poisoning was reached beyond which the antivenin was wholly without influence on the fatal result. This time relation of toxin-antitoxin neutralization is met with in diphtheria intoxication (Dönitz [8]) and tetanolysin hæmolysis (Madsen [9]).

## SUMMARY.

1. The action of different antivenins is highly, although not absolutely, specific for the venoms for which they are prepared.

[6] *Op. cit.*

[7] *Proc. Roy. Soc.*, Edinburgh, 1895, xx, 448.
See also *Brit. Med. Journal*, 1895, Pt. 2, 416.

[8] *Arch. intern. de Pharmacodynamie*, 1899, v, 425.

[9] *Zeitschr. f. Hyg.*, 1899, xxxii, 239.

The antivenomous effect is demonstrable by experiments on toxicity (*in vivo*) and on hæmolysis (*in vitro*).

2. Anticrotalus and antimoccasin sera possess therapeutic properties of high degree. By their employment before the stage of extreme prostration has been reached, poisoned guinea-pigs can be saved.

3. The standardization of antivenins must be made separately for the antitoxic and antihæmolytic actions, since these do not bear a constant and invariable relation to each other.

TABLE I.

A.—CROSS ANTITOXIC ACTION.

| | Protected against | | |
|---|---|---|---|
| | Crotalus venom | Water moccasin venom | Cobra venom |
| Anticrotalus 2.5 c.c. | 12 m.l.d. | 1 m.l.d. | 0 |
| Antimoccasin 2.5 c.c. | 3 m.l.d. | 5 m.l.d. | 0 |
| Anticobra 2 c.c. | 0 | 1 m.l.d. | 5 m.l.d. |

0 = no protection.

B.—CROSS ANTIHÆMOLYTIC ACTION.

| | Protected against | | | | |
|---|---|---|---|---|---|
| | Crotalus | Cobra | Moccasin | Daboia | Trimeresurus |
| Anticrotalus 1 c.c. | 10 m.h.d.* | 0.75 m.h.d. | 1.33 m.h.d. | 1.5 m.h.d. | 2.25 m.h.d. |
| Antimoccasin 1 c.c. | 4.125 m.h.d. | 4 m.h.d. | 40 m.h.d. | 4 m.h.d. | 5 m.h.d. |
| Anticobra 1 c.c. | 0 | 2 m.h.d. | 0 | 0 | 0.3 m.h.d. |

* M.h.d. = minimal complete hæmolytic dose for 8 c.c. of 2.5% suspension of defibrinated dog blood in 0.9% saline solution read after 2 hours' incubation at 37° C.

0 = no protection.

TABLE II.

Therapeutic experiments with anticrotalus serum.

Unfiltered Crotalus venom and anticrotalus goat serum filtered through a Chamberland bougie.

Test animals: guinea-pigs of 280 grams; injected intraperitoneally, April 20, 1904.

| Venom in grams | Symptoms before the injection of antivenin | Antivenin | | Symptoms after the injection of antivenin | Result |
|---|---|---|---|---|---|
| | | Time after injection of venom | Dose | | |
| 0.001 | Immediate irritating effect.<br>20 minutes: Abdominal tension steadily increasing; unable to walk. Gentle handling causes pain and cries; hair ruffled.<br>30–60 minutes: High degree of abdominal tension; wall presents dark purplish color. No resistance to handling, but animal still able to stand<br>1½ to 2 hours: Symptoms described increase gradually.<br>3 hours: Animal down; labored respirations; subnormal temperature. Died. | | 0 | | ⊹ 3 h. 48 m. |
| 0.001 | Symptoms similar to preceding but collapsed more slowly; able to stand until 4 hours 17 minutes after injection. | | 0 | | ⊹ 4 h. 34 m. |
| 0.001 | 50 minutes: Abdominal tension high; wall discolored by extravasation of blood; still able to stand, but unable to run. | 1 hour | 1 c.c. | 15 minutes: Very sick. 30 minutes: Condition unchanged, but later no increase in symptoms. A hæmo-serous fluid escaped from the needle hole.<br>1 hour: No change.<br>2 hours: Condition steadily improving.<br>4 hours: Able to run, but abdominal tension still remains high.<br>7 hours: Somewhat sick.<br>12 hours: Eats and runs about; local discharge drying up.<br>24 hours: Normal except for slight abdominal tension; weight 270 grams.<br>2 days: Well, weight 280 grams. | § |

| Venom in grams. | Symptoms before the injection of antivenin | Antivenin. | | Symptoms after the injection of antivenin. | Result. |
|---|---|---|---|---|---|
| | | Time after injection of venom. | Dose. | | |
| 0.001 | 1 hour and 50 minutes. Symptoms grave, scarcely able to stand; heart weak and quick. | 2 hours | 1 c.c. | 15 minutes: Condition unchanged.<br>4 hours: Critically ill, and fell.<br>Died 4 hours and 30 minutes after the injection of antivenin. | + 6 h. 30 m. (or 4 h. 30 m. after the injection of antivenin.) |
| 0.001 | 1 hour and 50 minutes. Symptoms similar to preceding. | 2 hours | 2 c.c. | 1 hour and 30 minutes: Condidition unchanged.<br>2 to 3 hours: Steady improvement.<br>18 hours: No more general symptoms; abdominal tension reduced, animal runs.<br>2 days: Complete recovery, weight 290 grams. | § |
| 0.001 | 2 hours and 50 minutes: Unable to move; critically ill. | 3 hours | 1 c.c. | 40 minutes: Condition unchanged.<br>80 minutes: Bloody fluid escaped from the needle puncture. Died 2 hours and 5 minutes after injection. | + 5 h. 5 m (or 2 h. 5 m. after the injection of antivenin.) |
| 0.001 | 2 hours and 50 minutes: Symptoms similar to preceding. | 3 hours | 4 c.c. | 2–3 hours: Condition slowly improved.<br>24 hours: Hæmo-serous fluid escaped from the needle hole. Abdominal tension still somewhat high; wall is of dark purple color. Able to run and eat. Weight 250 grams.<br>2 days: No general symptoms; local condition improving.<br>4 days: Well; weight 270 grams. | § |
| 0001 | 3 hours and 50 minutes: Symptoms similar to preceding. | 4 hours | 4 c.c. | 30 minutes: Seriously ill.<br>2 to 3 hours: No change. Died. | + 8 h. 45 m (or 4 h. and 45 m. after injection of antivenin.) |
| 0.001 | Symptoms similar to preceding. | 4 hours | 8 c.c. | General improvement after 8 hours.<br>Next morning well.<br>Recovery in 3 days; no complication after many months. | § |

TABLE III.

Therapeutic experiments with antimoccasin serum.
Water moccasin venom and filtered antiserum from goat.
Guinea-pigs of 420 grams; intraperitoneal injection, June 9, 1904.

| Venom in grams. | Symptoms before the injection of antivenin. | Antivenin. | | Symptoms after the injection of antivenin. | Result. |
|---|---|---|---|---|---|
| | | Time after injection of venom. | Dose. | | |
| 0.0024 | Immediate irritating effect.<br>15 to 20 minutes: Abdominal tension increasing steadily.<br>30 to 45 minutes: Hair erect; dyspnœic; unable to run.<br>1 hour: Marked dyspnœa and weakness; still slight resistance to handling.<br>2 hours: Unchanged.<br>3 hours: Unable to move; marked dyspnœa; paresis.<br>4 hours: Quick and shallow respiration; lies on side after 4 hours and 25 minutes.<br>Died 4 hours and 40 minutes after the injection. | | 0 | | † 4 h. 40 m. |
| 0.0024 | Similar symptoms to preceding. Died. | | 0 | | † 4 h. 35 m. |
| 0.0024 | Similar symptoms to preceding, died of dyspnœa and paralysis. | | 0 | | † 4 h. 20 m. |
| 0.0024 | Similar symptoms to preceding, but quicker collapse. | | 0 | | † 3 h. 50 m. |
| 0.0024 | Very weak just before injection; still able to move; high abdominal tension. | 1 hour | 1 c.c. | Animal gradually worse; down after 9 hours. Dyspnœa. | † 10 h. |
| 0.0024 | Similar symptoms to preceding. | 1 hour | 2 c.c. | Improvement commenced after 3 hours and became more rapid after 10 hours.<br>Next morning animal well, eats and runs.<br>No loss of weight. No complication followed the experiment. | § |
| 0.0024 | Marked dyspnœa, paresis, unable to run. | 2 hours | 2 c.c. | General symptoms became steadily more serious. Died. | † 3 h. 35 m. |

| Venom in grams. | Symptoms before the injection of antivenin. | Antivenin. | | Symptons after the injection of antivenin. | Result. |
|---|---|---|---|---|---|
| | | Time after injection of venom. | Dose. | | |
| 0.0024 | Similar condition to preceding. | 2 hours | 3 c.c. | No improvement; died of respiratory paralysis. | ⊹ 6 h. 20 m. |
| 0.0024 | Similar condition to preceding. | 2 hours | 4 c.c. | Improvement after 2 hours and animal runs after 6 hours. Next morning animal well Regained weight in 5 days. | § |
| 0.0024 | About same as above, but more dyspnœic. | 2 hours | 6 c.c. | Improvement after 2 hours; much better in 5 hours. 10 hours later animal runs. Next morning animal well. Weight regained in 4 days. | § |
| 0.0024 | Dyspnœic, atonic, unable to walk, abdominal tension high. | 3 hours | 4 c.c. | No improvement. | ⊹ 4 h. 10 m. |
| 0.0024 | Same condition as above. | 3 hours | 8 c.c. | Improvement started after 10 hours; animal weak after 24 hours. Regained weight in 2 days. No complications in many months. | § |
| 0.0024 | Marked dyspnœa and abdominal tension, unable to move. Critically ill. | 4 hours | 10 c.c. | Much improved after 10 hours. Next morning, well. No loss of weight. | § |

## TABLE IV.

Summary of therapeutic experiments.

The number of minimal lethal doses employed was 2, namely, 0.001 gram of Crotalus adamanteus and 0.0024 gram of water moccasin venom.

| Anticrotalus serum. | | | Antimoccasin serum. | | |
|---|---|---|---|---|---|
| Time. | Dose. | Result. | Time. | Dose. | Result. |
| Control | 0 | ⊹ 3 h. 48 m. | Control | 0 | ⊹ 4 h. 40 m. |
| | 0 | ⊹ 4 h. 34 m. | | 0 | ⊹ 4 h. 35 m. |
| | 0 | ⊹ 4 h. 20 m. | | 0 | ⊹ 4 h. 20 m. |
| | 0 | ⊹ 4 h. 50 m. | | 0 | ⊹ 3 h. 50 m. |
| 1 hour | 1 c.c. | § | 1 hour | 1 c.c. | ⊹ 10 h. |
| | | | | 2 c.c. | § |
| 2 hours | 1 c.c. | ⊹ 6 h. | 2 hours | 2 c.c. | ⊹ 3 h. 35 m. |
| | 2 c.c. | § | | 3 c.c. | ⊹ 6 h. 20 m. |
| | | | | 4 c.c. | § |
| 3 hours | 1 c.c. | ⊹ 5 h. | | 6 c.c. | § |
| | 4 c.c. | § | 3 hours | 4 c.c. | ⊹ 4 h. 10 m. |
| | | | | 8 c.c. | § |
| 4 hours | 4 c.c. | ⊹ 9 h. | 4 hours | 10 c.c. | § |
| | 8 c.c. | § | | | |

⊹ = Death.

§ = Recovery.

ADDENDUM.

A brief account of the foregoing experiments was read before the British Medical Association, at Oxford, July 28, 1904. I found, on my return to America, that Dr. George Lamb had turned his attention to this subject of the specificity of antivenin. Dr. Lamb employed several kinds of venom (Hoplocephalus, Daboia, etc.) and cobra and daboia antivenins, and he concluded that their actions are highly specific. In March 1905 Dr. Flexner received from Dr. Lamb several small vials of antidaboia serum together with a liberal sample of the venom, through which I was given the opportunity to test their reciprocal action against Crotalus venom and antivenin. The accompanying protocols show that the action of each of these venoms and antivenins is highly if not absolutely specific.

Guinea-pigs of 220 to 250 grams were injected intraperitoneally with venom or antivenin-venom mixtures. The venom solutions were freshly made, and the length of contact of venom and antivenin before injection was two hours at 37° C.

The toxicity of daboia venom was found to be as follows: doses of 0.0006 to 0.0001 gram killed guinea-pigs in about six hours; 0.00005 gram in nine hours; 0.000045 gram caused much loss of weight, but was not fatal; 0.00002 gram produced no symptoms. Hence 0.00005 gram of daboia venom contained one m.l.d. for guinea-pigs of 200 grams.

In neutralizing with the antidaboia serum the following results were obtained: 0.0004 gram daboia venom (= 8 m.l.d.) was completely neutralized by 0.5. c.c. of the antivenin; 0.2 c.c. of antivenin was not sufficient to neturalize the venom. The mixture of 0.1 c.c. of the serum with 0.0004 gram of venom was insufficient to reduce its toxicity to a subminimal lethal dose, but contained less than two m.l.d., as half of the mixture caused only a slight intoxication.

In another series of experiments 0.5 c.c. of antidaboia serum was added to 0.002 gram (40 m.l.d.) of the venom, after which the number of m.l.d. still present in the mixture was determined. It was found that the whole mixture contained a dose which produced death in twelve hours; half of the mixture, in

twenty hours; one third caused no symptoms. Therefore it can be stated that 0.5. c.c. of antidaboia serum reduced the number of m.l.d. from forty to between two and three.

The action of antidaboia serum upon Crotalus venom was studied to determine its specificity. Intraperitoneal injections of 0.0005 gram Crotalus venom killed guinea-pigs of 200 grams weight in seventeen hours; 0.00045 gram was not fatal.

To 1 c.c. of antidaboia serum, capable of neutralizing nearly 75 m.l.d. of daboia venom, were added amounts of Crotalus venom representing one, two, three, four, five, six, and eight m.l.d. The mixtures after two hours' contact were injected into the guinea-pigs. The results may be summarized as follows:

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Antidaboia serum | 1 c.c. | Crotalus venom | 0.0005 | (1 m.l.d.) | Survived | | | | | |
| " | " | " | 0.001 | (2 m.l.d.) | " | | | | | |
| " | " | " | 0.0015 | (3 m.l.d.) | " | | | | | |
| " | " | " | 0.002 | (4 m.l.d.) | Died in | 40 | hours | | | |
| " | " | " | 0.0025 | (5 m.l.d.) | " | " | 9 | " | | |
| " | " | " | 0.003 | (6 m.l.d.) | " | " | 12 | " | | |
| " | " | " | 0.004 | (8 m.l.d.) | " | " | 12 | " | | |

Autopsies upon the dead animals showed marked hæmorrhage in thorax, abdomen, and diaphragm. Hence 1 c.c. of antidaboia serum neutralized only about 3 m.l.d. of Crotalus venom, thus showing its highly specific nature.

For testing the antihæmolytic action of antidaboia serum 3 per cent. defibrinated dog blood, in 0.9 per cent. NaCl solution, was employed. Into each tube 8 c.c of the suspension were measured, to which the desired quantity of venom solution was added. The venom-antivenin mixture was kept for two hours at 37° C. before testing. Before reading the results the mixtures were kept at a temperature of 37° C. for two hours and at 20° C. for four hours.

The complete hæmolytic dose of daboia venom was found to be 0.0035 c.c. of 0.4 per cent. daboia venom solution per 8 c.c. of 3 per cent. defibrinated dog blood suspension. The m.h.d. of Crotalus venom was 0.015 c.c. of a 0.4 per cent. solution under the same conditions. It was found further that 1 c.c. of this daboia antivenin neutralized completely 0.1 c.c. 0.4 per cent. daboia venom solution, and only 0.035 c.c. of 0.4. per cent.

Crotalus venom solution. Hence the antihæmolytic action of the antivenin is highly specific.

Antidaboia serum 1 c.c. neutralized daboia venom 28.57 m.h.d.
" " " " Crotalus " 2.3 "

This serum also showed feeble antihæmolytic power against moccasin venom, but no power against cobra venom.