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TOXINS AND ANTITOXINS—SNAKE VENOMS AND ANTIVENINS.

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In this article we shall give an account of some quantitative determinations of the relation between the different snake venoms and their specific antivenins. The venoms employed were those of the cobra (*Naja tripudians*), of *Crotalus adamanteus* and of *Ancistrodon piscivorus.* Dr. George Lamb of India has kindly furnished us with cobra venom. The other two were personally procured by one of us (N.). The cobra antivenin was obtained through the kindness of Professor Calmette of Lille. The other two antivenins were prepared at this Institute.

McFarland[1] was the first to attempt the preparation of crotalus antivenin. In this, it appears, he had considerable difficulty, because the animal he used, the horse, reacted to subcutaneous injection with widespread œdema and inflammation. By the use of intravenous injection of unaltered venom McFarland obtained an antivenin which, according to his account, was protective against neuro-toxin, but had scarcely any effect on the irritative principle.

In order to avoid the inherent difficulty of immunization with unmodified venom, Flexner and Noguchi[2] attempted to eliminate the local effects by the use of a dilute solution of hydrochloric acid and of trichloride of iodine. They obtained in this way, in experiments on dogs and rabbits, an antivenin which neutralized perfectly the toxic effects of all the elements of crotalus venom.

In this Institute a *goat* was used, which was injected with unmodified crotalus venom subcutaneously. The animal stood the injection well; œdema was slight, and had disappeared at the end of a few days after each injection. The general condition of the

[1] McFarland, *Proc. of the Soc. of Amer. Bact.*, Dec., 1900.

[2] Flexner and Noguchi, *Jour. Med. Research*, 1903, vi, 363.

animal was apparently unchanged, and its weight slowly increased. (See Table I.)

It will be seen from reference to the protocol of the experiment that the highest antitoxic power was found on March 23 (see Table II) when one cubic centimeter of the serum neutralized the effects of about six minimal lethal doses. A little later the amount of the antitoxin diminished in spite of injections of relatively large doses of the venom. The antitoxin used in the following experiments was obtained on March 30 and 31, 1904.

On May 11, 1904, two kids were born and the amount of antitoxin in the colostrum was determined. The quantity of antitoxin was about the same both in the blood and in the milk. This fact is of considerable interest inasmuch as previous experiments[3] had shown that the amount of antitoxin in the milk was much less than that in the blood. The milk of non-immunized goats was also examined as controls and was found to be devoid of antivenomous action.

Experiments on immunization against the venom of the *water moccasin* were first undertaken with *unmodified* venom injected *subcutaneously*. The goats appeared to stand the injections of small amounts very well, but in two cases (Tables III and IV) the animals died after the injection of 0.2 gram. They had previously tolerated repeated injection of smaller quantities.

To determine whether 0.2 gram was the m. l. d. for goats of this size, 0.15 gram of moccasin venom was injected subcutaneously into a normal goat. But, as shown in the protocol of the experiment (Table V), this animal died after a single subcutaneous injection of 0.15 gram. Then we used the moccasin venom *modified* by placing 0.5 gram of the dried venom, dissolved in one hundred cubic centimeters of one per cent. hydrochloric acid, in the thermostat at 37° C. for twenty-four hours. The solution was neutralized with sodium hydroxide. Immunization with this modified venom was rapidly and easily accomplished (Table VI). A trial serum, drawn on May 13, 1904, showed a weak antivenomous power, but after continued injections of the venom the antitoxic

[3] Salomonson and Madsen, *Ann. de l'Inst. Pasteur*, 1897, xi, 315, and 1899, xiii, 262.

standard rose to a point at which, on May 30, 1904, one cubic centimeter of the serum completely neutralized all effects of 0.0024 gram (2 m. l. d.) of the unmodified venom (Table VII). This antivenin was used in the experiments which are to be discussed later.

Solutions of the venoms were made as follows:

0.5 grm. dried crotalus venom in 100 c.c. water,
0.5 grm. dried moccasin venom in 100 c.c. water,
0.4 grm. dried cobra venom in 100 c.c. water.

These solutions preserved under toluol at a temperature of from 2° to 3° C. were used for all the experiments which follow. The two first solutions showed no perceptible deterioration during the month of experimentation, but the toxicity of the cobra venom was sensibly diminished.

CROTALUS VENOM.

The determination of toxicity was generally made by intraperitoneal injections into guinea-pigs. Table VIII, presenting a small series of experiments, shows in detail the relation between the weight of the animal and the toxicity of the venom. Apparently this relation remains about the same per kilo for animals of from 250 to 500 grams, while smaller animals weighing about 125 grams are much more sensitive. Crotalus venom *passed through a Chamberland filter always loses more than fifty per cent. of its toxicity,* as shown in Table IX. The toxicity is considerably lower and the action is much more irregular when the venom is injected *subcutaneously* (Table X). White rats are very resistant to its action (Table XI). Tables XII and XIII give the details of experiments dealing with the *neutralization of crotalus venom by its specific antivenin.*

To 0.006 gram of venom were added the amounts of antivenin shown in the first column of Table XIII. The mixtures were kept two hours at 37° C. and then injected in the fractional quantities indicated in the second column of the table. Taking the dose which kills after from fifteen to seventeen hours as a unit, we have derived the facts shown in Table XIV, where under "*n*" are indi-

cated the quantities of antivenin added to a constant dose of venom, 0.006 gram, and under "q observed," the observed toxicity.

These observed values, after allowing for errors in experiment, can be expressed by the same formula, which represents the combinations of toxins and antitoxins of other substances.[4]

$$\frac{\text{Free toxin}}{\text{vol.}} \cdot \frac{\text{Free antitoxin}}{\text{vol.}} = K \cdot \text{Toxin-antitoxin.}$$

In this case:

$$\frac{1}{q_0}\left[n\frac{1}{q}\cdot p - \left(\frac{1}{q}-\frac{1}{q_0}\right)\right] = K\left(\frac{1}{q}-\frac{1}{q_0}\right)^2$$

where $1/q$ represents the quantity of antitoxin equivalent to an amount of toxin used and K the constant of association.

With $p = 1$ and $K = 0.0048$, we found the value of q, shown under "q calc. I." For greater convenience these results are graphically represented in Fig. 1. The tracing shows the calculated values of "q," while the dotted circle shows the observed values. It will be noted, therefore, that the values derived from the experiments mentioned above can be expressed according to the simple formula:

$$-\frac{\delta q}{dn} = Kq.$$

We have shown that this formula represents the action of saponin and cholesterin[5]; the column under "q calc. II" shows the correspondence.

The experiments on *rabbits* (Table XV) showed a marked difference between subcutaneous and intravenous injections of venom. For the *neutralization experiments* shown in Table XVI, intravenous injections only were used. The technique was the same as that used in the experiments with guinea-pigs, the quantity of venom being always 0.006 grm. The results obtained by taking as a unit of toxicity a dose killing after five days are summarized in Table XVII. They are shown in Fig. 1 by a dotted square. The form of the tracing obtained agrees to a certain extent with that

[4] Festkrift ved Indvielsen af Statens Serum Institut, Copenhagen, 1902.
[5] Extr. du Bul. de l'Acad. Royale de Danemark, 1904.

determined for guinea-pigs; further calculations were omitted, since the first part of the determination showed an error.[6]

Antivenin produced by immunization has also the power of

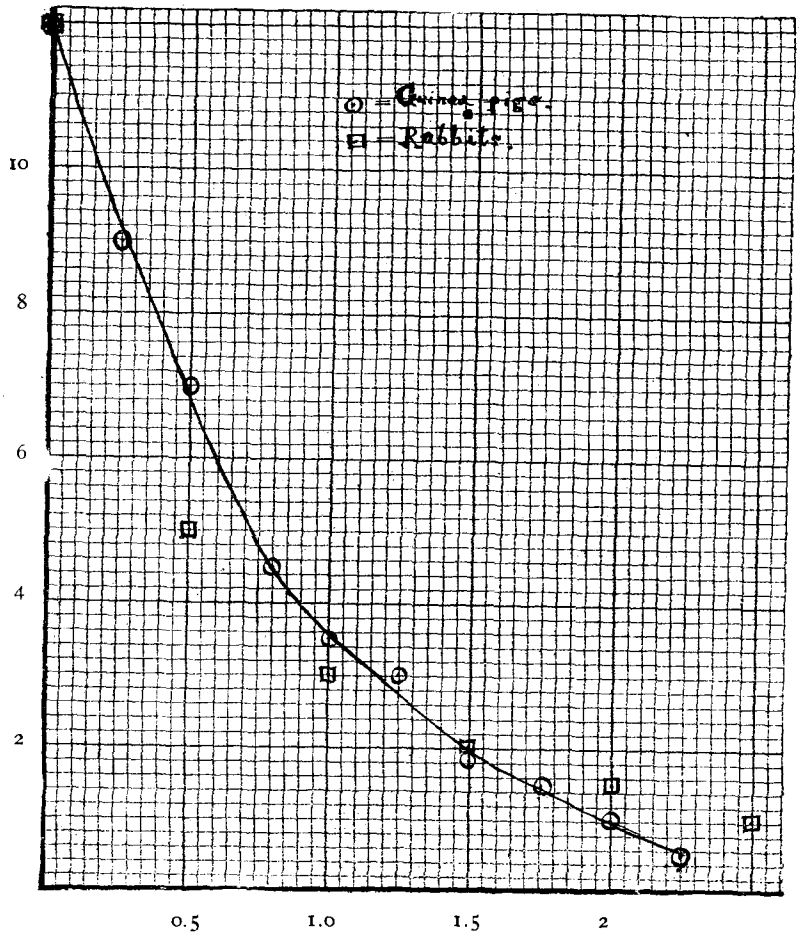

FIG. 1. Crotalus venom-antivenin.

neutralizing the hæmolytic property of crotalus venom. Table XVIII gives the details of experiments; one cubic centimeter of

[6] Perhaps these errors are due to the different methods of injection. At the time when these experiments were carried out, the researches of Morgenroth (*Berliner klin. Woch.*, 1904, xli, 526) on the slow combination of toxin and antitoxin of diphtheria had not been published. Nevertheless, it does not seem unlikely that the reaction will be finished after two hours at 37° C.

0.05 per cent. crotalus venom mixed with the quantities of crotalus antivenin indicated under "*n*" was added to 0.9 per cent. sodium chloride solution to bring the total volume to two cubic centimeters. These mixtures were kept two hours at 37° C. and then their

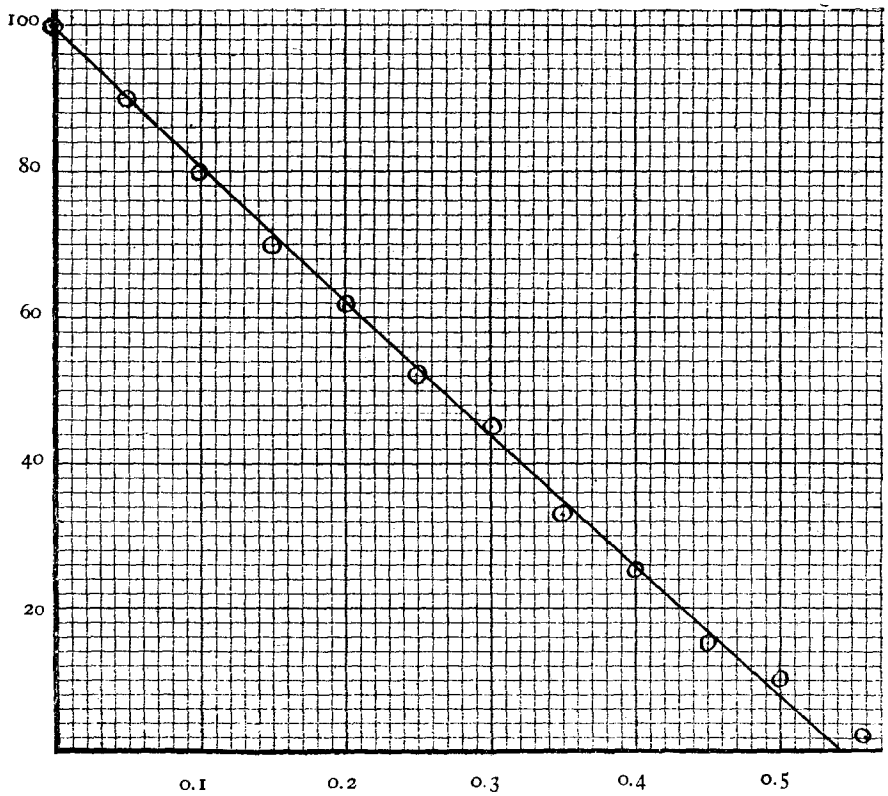

FIG. 2. Crotalus lysin-antilysin (dog's blood).

hæmolytic action, on a five per cent. suspension of dog's blood in 0.9 per cent. saline solution, was examined by the method used in this Institute. In Table XVIII, columns marked "I, II, III," show the doses producing equal hæmolysis; the averages, representing the hæmolytic power, have been estimated from these figures and are given under "*q* obs." As shown graphically in Fig. 2, the form of tracing obtained by neutralization is, in this case, practically a straight line.[7]

[7] The constant of equilibrium was, in this case, almost zero. In the table are shown, under "q calc.," the values estimated on the assumption that 1 c.c. of

COBRA VENOM.

The first determinations of the m. l. d. were made by subcutaneous injections of 0.2 per cent. solution of venom into *guinea-pigs* weighing from 650 to 670 grams. (Table XIX.) A little later another solution of venom, 0.4 per cent., filtered through a Chamberland filter (Table XX) was examined. A comparison was, however, somewhat difficult, because the animals of the two series were not

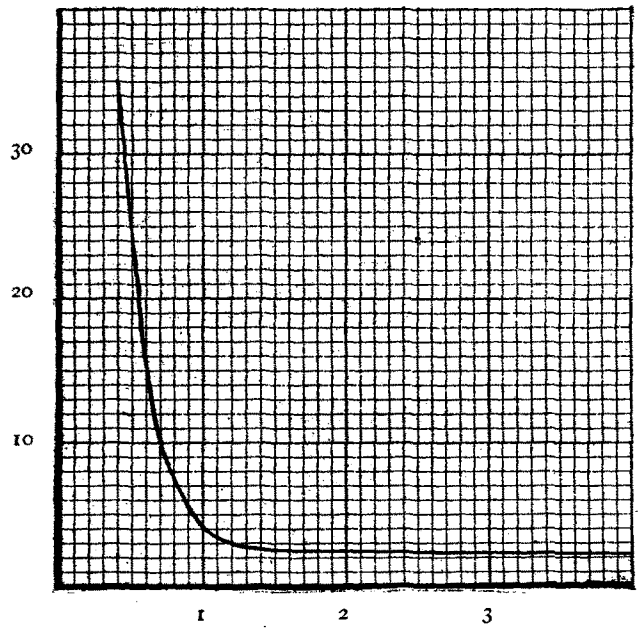

FIG. 3. Toxicity of cobra venom.

of equal weight; it was seen, nevertheless, that filtration did not perceptibly diminish the toxicity of the solution.

The relation between the dose and the toxicity is shown by the tables. In the first place, the time of death is greatly shortened up to a certain point as the dosage is increased. With 0.0005 gram it is 3.75 hours, but beyond this point an increase of the dose does

this antivenin is equivalent to 1.86 c.c. of the solution of crotalus venom. Moreover, for 0.55 c.c. of antivenin there was a discrepancy, pointing to a feeble dissociation of the venom-antivenin combination, corresponding practically to the value of "K" (the constant of dissociation) of 0.0006.

not proportionally shorten the time intervening between the injection and the death of the animal.

In Table XXI are recorded, for the greater part, the same experiments shown in Tables XIX and XX, undertaken, however, with a little larger animals (marked *). If it be arbitrarily assumed, as in a previous paper,[8] that 0.0005 gram is equal to one m. l. d., the results will group themselves with a certain regularity about this dose. Perhaps this will appear more clear by reference to Fig. 3, where the m. l. d. is drawn on the axis of the abscissa, while the time, in hours, is constructed on the ordinates. It will be seen that the determinations form a very regular curve of an asymptotical type. The decrease in time does not agree with the increase in dose; this decrease is perhaps due to the time of incubation and to the method of injection, since it is well known that venom requires some time for absorption from subcutaneous tissues. With intravenous injection the decrease would probably have been much more pronounced. It is evident that the values obtained with doses ranging from 0.6 to 1.2 m. l. d. give the most decisive determinations. The lower and very much larger doses show results which are much less certain. The method just described has, moreover, the advantage that it is not always necessary to find out the dose which will kill within a fixed time. After determining the scale once one can find by interpolation which fraction of the minimal lethal dose is present in a certain mixture.

On account of the very important rôle which lecithin plays in the hæmolysis caused by cobra venom, some experiments were undertaken in order to learn whether one cubic centimeter of a 1/50 *N* solution of lecithin would produce any appreciable change in the toxicity for guinea-pigs. As shown by Table XXII, no such change occurs.

The combination of cobra venom and its specific antivenin seems to occur at 36° C. very rapidly. The small series of experiments, shown in Table XXIII, does not indicate any appreciable difference in the neutralizing power, whether venom and antivenin are mixed together and injected immediately or injected at the end of six

[8] Arrhenius and Madsen, *Acad. Roy. des Sciences et des Let. de Danemark*, 1904.

hours.[9] The mixtures of toxin and antitoxin were incubated for two hours at 36° C.

During the experiments undertaken to explain the neutralization of venom with antivenin, it appeared that the first preparations of antivenin which were at our disposal were too feeble. In Table XXIV will be found in the second column, under "*n*," the quantities of serum (Antivenin I), in cubic centimeters, which were added

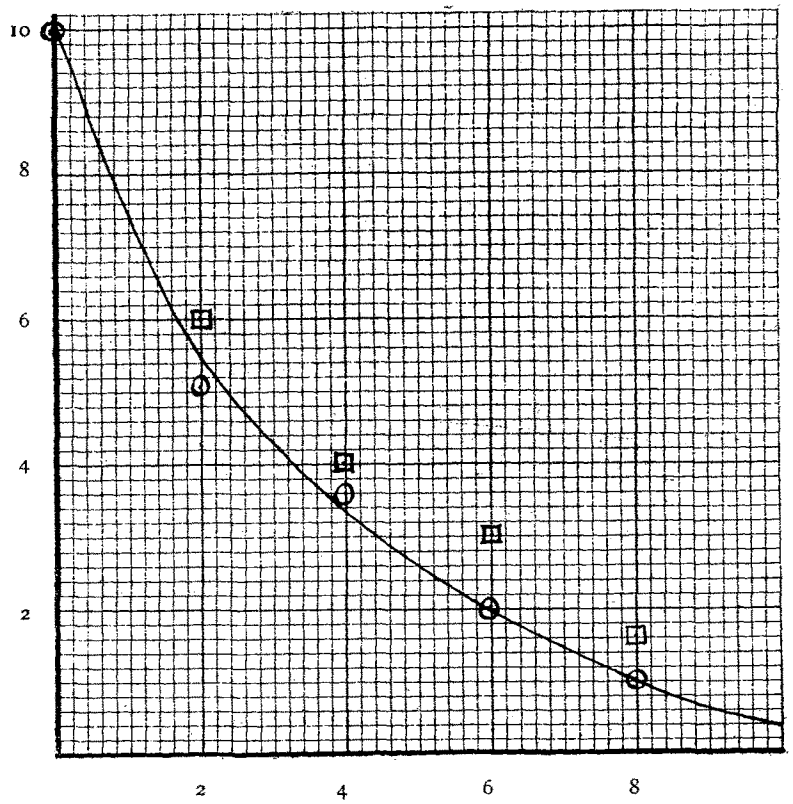

FIG. 4. Cobra venom-antivenin (guinea pigs).

to 0.008 gram (16 m. l. d.), and to 0.004 gram respectively of the venom. In the following column is shown the fraction of this

[9] The experiments were not complete. Experiments with intravenous injection were also lacking, and would be necessary to make it perfectly clear that the reaction between venom and antivenin is rapid (cf. the researches of Morgenroth on diphtheria antitoxin, *loc. cit.*)

mixture which was injected; and in the last two columns the results. One experiment with a new antivenin (II) showed that from ten to twelve cubic centimeters would neutralize the effect of 0.004 gram. It was necessary to know that this antivenomous effect was not due in part to the normal serum of the horse. In one ex-

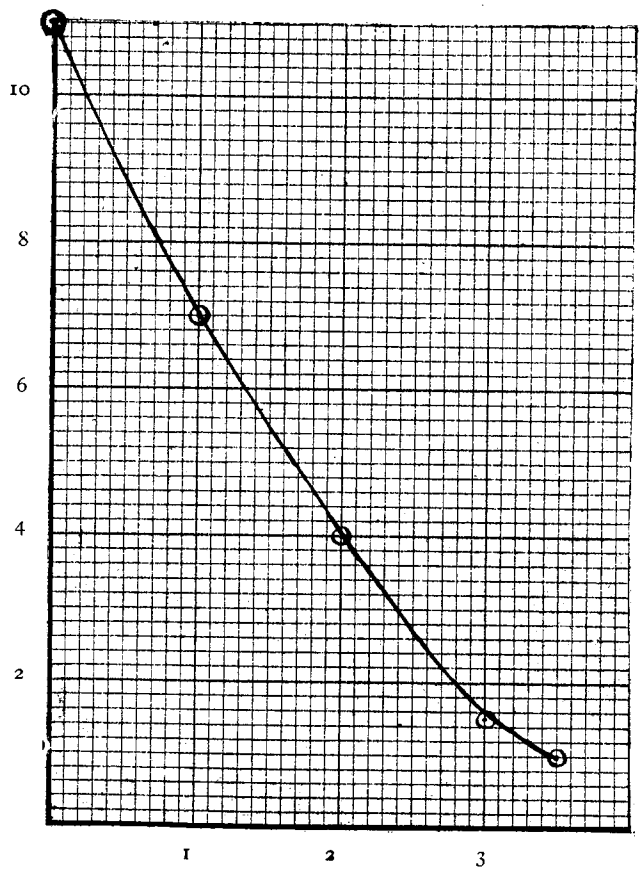

FIG. 5. Cobra venom-antivenin (guinea pigs).

periment twenty cubic centimeters of normal horse serum gave almost no antagonistic effect when tested against 0.008 gram of the venom (Table XXV). With this antivenin (II) (Table XXVI) some experiments upon partial saturation were undertaken. To 0.0028 gram of the venom were added the amounts of antivenin indicated under "*n*." The mixtures were, as usual, incubated

for two hours at 36° C.; then the fractions indicated in the following column were injected intraperitoneally into guinea-pigs. There were series of experiments, one with animals of 370 grams, another with those of 450 grams. The determinations of toxicity, q, in the last series (B) will be found in Table XX. They can be summarized as follows:

n	q
0	10
2	5
4	3.5
6	2
8	1.7
10	< 1

Fig. 4 furnishes a graphical representation of these results.

In one series (guinea-pigs weighing 370 grams) there was no determination made for $n=0$. The other values are marked by a dotted square in Fig. 4. It will be seen that this curve corresponds very well to the first one.

The last series of *neutralization* experiments were carried out in June, 1904, with an antivenin considerably stronger than that previously used (Table XXVII); four cubic centimeters of this antivenin neutralized effectively 0.003 gram of venom. The results of these experiments may be summarized as follows:

n	q
0	11
1	7
2	4
3	1.5 (?)
4	< 1

The tracing in Fig. 5, which graphically represents the results of neutralization, is not very strongly curved. The results of neutralization of the *hæmolysin of cobra venom* by its antivenin was tested twice with Antitoxins I and II. The procedure was that which we have usually employed: one cubic centimeter of a 0.1 per cent. solution of cobra venom was mixed with various quantities of antivenin; to these mixtures was further added a sufficient amount of physiological salt solution to bring the total volume to two cubic centimeters. A mixture, kept for two hours at 37°, was

measured into tubes and eight cubic centimeters of a one per cent. suspension of horse's blood corpuscles were rapidly added (each liter of the suspension contained eight cubic centimeters of 1/100N lecithin solution). The tubes were incubated for two hours at 37° C. and then over night at a lower temperature; hæmolysis was estimated by our usual method.

The figures in Table XXVIII are the averages, derived in the

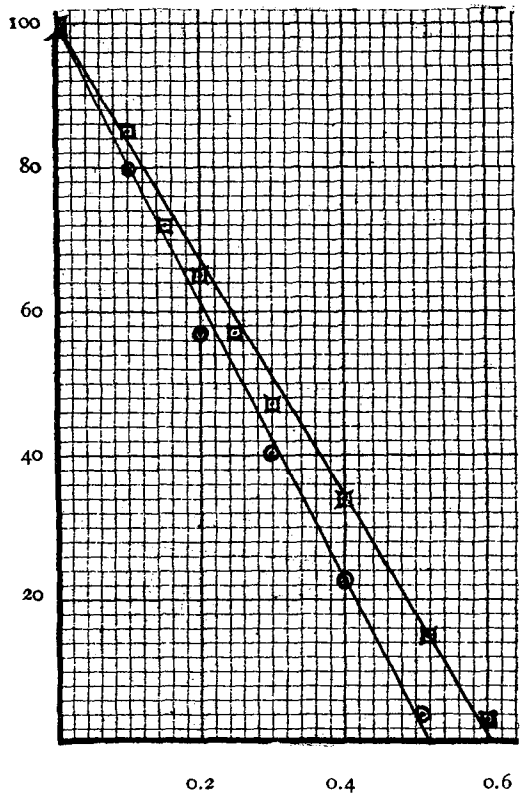

FIG. 6. Cobra lysin-antilysin.

same way as those of the experiments recorded in Table XVIII. In Fig. 6 the observations with Antivenin I are marked by a dotted circle, and with Antivenin II by a dotted square. The tracings, as may be seen, are rather close to straight lines.

The theoretical values of "q calc." are calculated under the sup-

position that one cubic centimeter of Antivenin I is equivalent to 1.98 c.c. of venom solution, and of Antivenin II equivalent to 1.7 c.c. of venom ($K=0$). For both series, the last values for "q obs." are then considerably lower than the calculated ones. This indicates that there is a clear dissociation[10] (corresponding to $K=0.0016$). Thus it is not quite correct to assume that the curve of neutralization of cobrolysin and of cobra antilysin is a completely straight line.[11]

WATER MOCCASIN VENOM.

The toxicity of water moccasin venom was examined on two occasions, May 6 and August 22, 1904; during the interval of one hundred and eight days the venom was preserved at from 2° to 4° C. The animals employed for these experiments were guinea-pigs weighing 260 grams. The venom was injected into the peritoneal cavity. A comparison between Tables XXIX and XXX shows that the toxicity was intact. The experiments upon the *neutralization of this venom by its specific antivenin* show singular results. They were carried out by mixing 0.012 gram of venom,—about 10 m. l. d.—with varying amounts of antivenin. After the mixture had been kept for three hours at 37° C. its toxicity was tested by injecting various fractions into guinea-pigs weighing 250 grams. (Table XXXI.) By the addition of 2 c.c. of serum the toxicity was lowered from 10 to 6; of 4 c.c., to about 4 or 5; of 5 c.c., to 3; and of 6 c.c., to about 2.5. But by the addition of still larger amounts of antivenin no further reduction of the toxicity could be produced, as experiments with 8, 9, 10, 20 and 40 c.c. of antivenin showed. This phenomenon was perhaps due to the fact that the antivenin in large enough doses had in itself a toxic action.[12] Unfortunately, our stock serum was exhausted, and we were unable to carry further an investigation of this phenomenon.

Finally, a study was made of the *hæmolytic* action of the venom of the water moccasin; one cubic centimeter of this venom (stock

[10] Myers, *Jour. Path. and Bact.*, 1900, vi, 415. Flexner and Noguchi, *Jour. Path. and Bact.*, 1903, viii, 379.

[11] Kyes, *Berl. klin. Woch.*, 1904, xli, 494.

[12] In fact, it is well known that the normal serum of the goat is often toxic for quinea-pigs.

solution) was mixed with different quantities of moccasin antiserum. The mixtures were incubated for two hours at 37° C. Special experiments had shown that the reaction was complete at the end of ten minutes. The hæmolytic tests were made in the usual way, using a five per cent. suspension of dog's blood. The results are given in Table XXXII and in Fig. 7. It will be seen

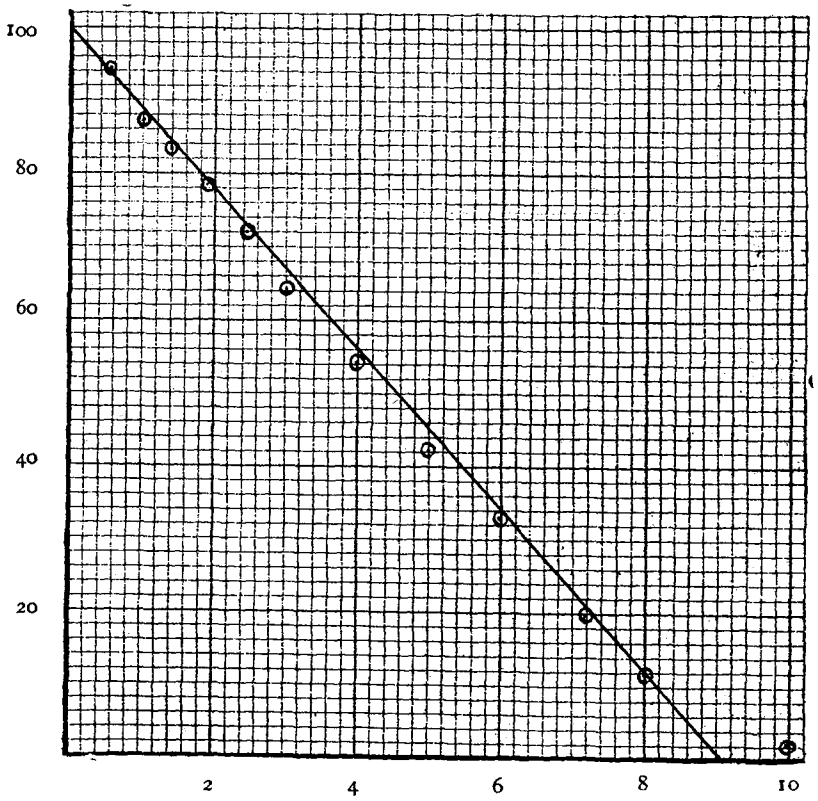

FIG. 7. *Ancistrodon piscivorus* lysin-antilysin.

that the tracing representing the combination is approximately a straight line, except for high concentrations of antitoxin. The values are estimated on the supposition that one cubic centimeter of antivenin is equivalent to 1.2 cubic centimeters of the venom solution, and that $K = 0$. In this case the values calculated for higher concentrations show more variation than in the case of crotalus and cobra lysins. K should be about 0.006.

Unfortunately, the lack of material prevented the continuation of our experiments. Those given are to be regarded as preliminary on account of their small number. For this reason we have not presented any general conclusions, but have given our experiments in the hope that they may prove the starting point of similar researches in the future.

SUMMARY.

A specific antivenin against crotalus venom can be prepared by the immunization of goats. A specific antivenin against water moccasin venom can be produced by the immunization of goats with this venom, modified by hydrochloric acid. Immunization with the unmodified venom is very difficult.

The toxicity of crotalus venom is diminished more than fifty per cent. by passage through a Chamberland filter. There is a simple relation between the toxicity and the body weight for guinea-pigs weighing from 250 to 500 grams. Smaller guinea-pigs (125 grams) are comparatively less resistant. The toxicity is smaller by subcutaneous than by intraperitoneal injection (guinea-pigs), or by intravenous injection (rabbits). White rats are very resistant.

The toxicity of cobra venom is not measurably diminished by filtration through a Chamberland filter. The relation between the amount of venom and the corresponding time of death is very regular, and can be expressed by a curve of asymptotic nature. Lecithin does not increase the toxicity.

The tracings representing toxin-antitoxin neutralization for the three venoms (crotalus, cobra and moccasin) show deviation from the straight line. This deviation is most pronounced for the toxic quota of the venoms. The tracing representing crotalus venom-antivenin neutralization, determined on guinea-pigs, can, within errors of experiment, be expressed by the equation:

$$\text{Free toxin} \cdot \text{Free antitoxin} = K \cdot \text{toxin-antitoxin}.$$

The corresponding tracing determined on rabbits is somewhat different, but both tracings are much more markedly curved than that for cobra venom-antivenin. The neutralization tracing of water moccasin venom shows the peculiarity, that small amounts of anti-

TABLE I

IMMUNIZATION OF A GOAT WITH CROTALUS VENOM.

Nov.	4, 1903,	0.0001 grm.	
	7	0.0002	
	10	0.0004	
	13	0.0008	
	16	0.0012	
	20	0.0016	
	23	0.002	
	26	0.0025	
	30	0.003	
Dec.	4	0.0035	
	9	0.004	
	12	0.004	
	16	0.005	
	21	0.008	
	26	0.01	
	31	0.015	
Jan.	2, 1904,	0.02	
	6	0.032	
	13	0.04	
	16	0.04	
	19	0.05	
	23	0.08	
	28	0.1	
Feb.	2	0.14	
	10	0.16	
	15	0.2	Slightly ill.
	23	0.05	Blood was drawn before the injection (1 c.c. of this serum protected against 2.5 m. l. d.).
	29	0.1	
Mar.	4	0.2	Slight swelling around the site of injection.
	9	0.25	
	15	0.3	
	19	0.35	
	23	0.4	Bled (1 c.c. protected against 6 m. l. d.).
	30		Bled about 350 c.c. (1 c.c. protected against 5 m. l. d.).
	31		Bled about 500 c.c. (1 c.c. protected against 5 m. l. d.).
Apr.	18	0.05	Bled (1 c.c. protected against 0.4 m. l. d.).
	20	0.1	
	24	0.15	
	27	0.2	
	30	0.35	
May	6		Bled (1 c.c. protected against 4 m. l. d.).
	7		Bled (1 c.c. protected against 3.2 m. l. d.).
	11		Two kids born. The milk was tested and it was found that 1 c.c. protected against 2.4 m. l. d.

venin decrease the toxicity to a minimum, but the toxicity is again increased by further addition of antitoxin.

The tracing representing neutralization of the hæmolysins of the three venoms are different from the tracings of neutralization of the toxins, and approach very closely to a straight line. Still, in all instances, the determinations with great concentrations of antilysin show pronounced deviation, perhaps due to some dissociation of the toxin-antitoxin combination.

TABLE II.

TESTS OF THE ANTIVENOMOUS POWER DURING IMMUNIZATION.

The animal was bled on February 23, 1904. Guinea-pigs of 500 grm. were used for the experiment.

Serum (antivenin) 2 c.c. + crotalus venom 0.002 grm. = No symptoms.
Serum (antivenin) 2 c.c. + crotalus venom 0.004 grm. = Sick for 3 days.
Serum (antivenin) 2 c.c. + crotalus venom 0.005 grm. = Sick for 2 days; recovered.
Serum (antivenin) 2 c.c. + crotalus venom 0.0075 grm. = Death in 27^h 7^m.
Serum (antivenin) 2 c.c. + crotalus venom 0.01 grm. = Death in 5^h 45^m.

The animal was bled on March 23, 1904. Guinea-pigs of 400 grm. were used.

Serum 2 c.c. + crotalus venom 0.0075 grm. = No symptoms.
Serum 2 c.c. + crotalus venom 0.01 grm. = Sick for 1 day; recovered.
Serum 2 c.c. + crotalus venom 0.0125 grm. = Death in 3^h 35^m.

The animal was bled on March 30 and 31, 1904. Guinea-pigs of 280 grm. were used.

Serum 2.5 c.c. + crotalus venom 0.005 grm. = No symptoms.
Serum 2.5 c.c. + crotalus venom 0.006 grm. = No symptoms.
Serum 2.5 c.c. + crotalus venom 0.0075 grm. = Death in 12^h.
Serum 2.0 c.c. + crotalus venom 0.006 grm. = Death in 16^h.
Serum 4.0 c.c. + crotalus venom 0.01 grm. = Death in 17^h.

The animal was bled on April 18, 1904. Guinea-pigs of 240 grm. were used.

Serum 2.5 c.c. + crotalus venom 0.0015 grm. = No symptoms.
Serum 2.5 c.c. + crotalus venom 0.003 grm. = Death in 12^h.

The animal was bled on May 6, 1904. Guinea-pigs of 290 grm. were used.

Serum 2.5 c.c. + crotalus venom 0.005 grm. = Slightly ill for 1 day; recovery.
Serum 2.5 c.c. + crotalus venom 0.006 grm. = Death in 12^h.

The animal was bled on May 7, 1904. Guinea-pigs of 290 grm. were used.

Serum 2.5 c.c. + crotalus venom 0.004 grm. = No symptoms.
Serum 2.5 c.c. + crotalus venom 0.005 grm. = Death in 7^h.
Serum 2.5 c.c. + crotalus venom 0.006 grm. = Death in 12^h.

The animal was bled on May 11, 1904. Guinea-pigs of 290 grm. were used.

Serum 2.5 c.c. + crotalus venom 0.003 grm. = Slightly ill; recovery.
Serum 2.5 c.c. + crotalus venom 0.004 grm. = Death in 12^h.

The milk of the same goat was taken several hours after the delivery.

Colostrum 2.5 c.c. + crotalus venom 0.003 grm. = Slightly ill; recovery.

TABLE III.

IMMUNIZATION OF A GOAT WITH THE VENOM OF WATER MOCCASIN BY SUBCUTANEOUS INJECTION.

Nov.	7, 1903,	0.0001 grm.,	no symptoms.
	10	0.0002	
	13	0.0004	
	16	0.0008	
	20	0.0012	
	23	0.0016	
	26	0.002	
	30	0.0025	
Dec.	4	0.003	
	9	0.004	
	12	0.004	
	16	0.005	
	21	0.008	
	26	0.01	
	31	0.015	
Jan.	2, 1904,	0.02	
	6	0.032	Small abscess at the site of injection.
	13	0.04	
	16	0.04	
	19	0.05	
	23	0.08	
	28	0.1	
Feb.	2	0.14	
	10	0.16	
	15	0.2	Ill after 24 hours.
	20		Very ill, but no local symptoms; dyspnœa, loss of appetit: and unable to stand up.
	21.		All symptoms above described increasing.
	22.		Died during the foregoing night.

TABLE IV.

IMMUNIZATION OF A GOAT WITH THE VENOM OF WATER MOCCASIN BY SUBCUTANEOUS INJECTION.

Feb.	29, 1904,	0.01	grm.
Apr.	4	0.025	No symptoms.
	9	0.04	
	15	0.07	
	19	0.09	
	23	0.12	A large abscess formed at the point of injection.
May	2	0.14	Quite well.
	6	0.18	
	9	0.2	Death after 18 hours.

TABLE V.

TOXICITY OF THE VENOM OF WATER MOCCASIN FOR A GOAT WEIGHING 27 KILO.

On April 10, 1904, at 11:43 A. M. 0.15 grm. (in 5 c.c.) of the venom of water moccasin was injected at the right side of the back, subcutaneously. After from three to four hours the animal became very inactive, but was still able to stand. It avoided lying down or moving. At the same time the whole of the injected side became intensely swollen and very painful. At the end of ten hours after the injection the animal was still able to support its body on the legs. The swelling at the site of injection became more marked and over the whole body there was swelling and œdema in varying degree, but more intense in the region of the injection. The skin around the needle puncture showed no necrosis. Death ensued at the end of twenty-two hours after injection, with symptoms of great dyspnœa.

Autopsy was performed one hour after death. There was no rigor mortis. There was enormous swelling of the entire cadaver, especially pronounced over the abdominal wall and the right back, which was very œdematous. A large quantity of bloody exudate had accumulated in the subcutaneous tissues and the exudate had a jelly-like consistence. The muscles had a dark-purple or deep blackish color, the muscles along the spinal column being darkest. The muscles were friable and very easily torn into pieces. They were infiltrated with extravasated blood corpuscles as well as with free hæmoglobin. The peritoneum, omentum, and serous membranes of the pleuræ and pericardium were free from hæmorrhage. The hæmorrhage in the subcutaneous tissue did not extend into the cervical region. Two dead fœtuses found in the uterus presented marked rigor mortis, but almost no hæmorrhage.

TABLE VI.

IMMUNIZATION WITH THE VENOM OF WATER MOCCASIN MODIFIED BY HYDROCHLORIC ACID.

Dried venom 0.5 grm.; 1 per cent. aqueous solution of hydrochloric acid 100 c.c. The mixture was kept 24 hours at 37° C.

After incubation for 24 hours at 37° C., the mixture was neutralized with hydrate of sodium, which produced a bulky precipitate of the proteids of the venom. A goat was injected subcutaneously.

Apr.	13, 1904,	0.025 grm.	
	18	0.025	
	20	0.04	
	22	0.05	
	24	0.07	
	26	0.1	
	28	0.2	
	30	0.2	
May	2	0.3	
	4	0.4	
	6	0.7	
	13		Bled.
	14	0.4	
	18	0.2	
	21	0.3	
	24	0.5	
	30		Bled (600 c.c.).

TABLE VII.

TESTS FOR ANTIVENIN DURING IMMUNIZATION.

Serum was drawn on May 13, 1904. Guinea-pigs of 290 grm. were used.
Serum 2 c.c. + moccasin venom 0.0036 grm. = Death in 7^h and 20^m.
Serum 2 c.c. + moccasin venom 0.006 grm. = Death in 2^h.
Serum 2 c.c. + moccasin venom 0.008 grm. = Death in 1^h and 50^m.
Serum 2 c.c. + moccasin venom 0.012 grm. = Death in 40^m.
Serum was drawn on May 30, 1904. Guinea-pigs of 400 grm. were used.
Serum 2 c.c. + moccasin venom 0.012 grm. = Death in 36^h.
Serum 2 c.c. + moccasin venom 0.012 grm. = Death in 1^h and 30^m.
Serum 2.5 c.c. + moccasin venom 0.006 grm. = Recovered.
Serum 2.5 c.c. + moccasin venom 0.008 grm. = Death in 10^h.
Serum 2.5 c.c. + moccasin venom 0.01 grm. = Death in 4^h 10^m.
Serum 2.5 c.c. + moccasin venom 0.012 grm. = Death in 3^h 40^m.

TABLE VIII.

RELATION BETWEEN THE BODY WEIGHT AND SUSCEPTIBILITY OF GUINEA-PIGS AFTER INTRAPERITONEAL INJECTION OF CROTALUS VENOM (NOT FILTERED).

Guinea Pigs of 500 grm.		Guinea Pigs of 250 grm.		Guinea Pigs of 125 grm.	
Venom in grm.	Result.	Venom in grm.	Result.	Venom in grm.	Result.
0.0012	+ 9^h 25^m	0.0006	+ 10^h 10^m	0.0003	+ 9^h 5^m
0.001	+ 18^h	0.0005	+ 18^h	0.00025	+ 6^h
0.0008	+ 12^h 25^m	0.0004	§ very ill	0.0002	+ 12^h
0.0008	§	0.0004	§	0.0002	+ 5^h 30^m
0.0008	§	0.0003	§	0.00015	+ 9^h
0.0008	§			0.0001	§ sick
				0.00008	§

+ = Death. § = Recovery.

TABLE IX.

DETERMINATION OF THE MINIMAL LETHAL DOSE OF CROTALUS VENOM FILTERED TROUGH THE CHAMBERLAND BOUGIE.

The stock solution was made as follows: Dried venom, 0.5 grm. and distilled water, 100 c.c. Guinea-pigs weighing 250 grm. were used and the venom was administered intraperitoneally on April 5, 1904.

Venom in grm.	Result.	Remarks.
0.0002	§	Almost no symptoms; the abdominal tension was slightly increased for one day.
0.00025	§	Ditto.
0.0003	§	Ditto.
0.00035	§	Sick for one day; the abdominal tension was much increased the first day.
0.0004	§	Ditto.
0.0005	§	Very sick for the first day; the abdominal tension was enormously increased during two days.
0.0006	§	Ditto.
0.001	§	Very sick for two days; had a very high abdominal tension; after 3 days improved rapidly.
0.0012	+ 15^h	
0.0015	+ 10^h	

+ = Death. § = Recovery.

TABLE X.

DETERMINATION OF THE MINIMAL LETHAL DOSE OF CROTALUS VENOM WITHOUT FILTRATION.

The stock solution was made as follows: Dried venom 0.5 grm.; distilled water 100 c.c. Guinea-pigs of 320 grm. were injected subcutaneously, April 21–23, 1904.

Venom in grm.	Result.	Remarks.
0.0035	+ 14 days	First day, very sick; second day, the site of injection highly swollen and the hairs came off about the site of needle puncture; the skin was softened and a small amount of a sero-sanguineous fluid discharged from the needle puncture, but the animal seemed more active than on the first day. The appetite was normal. The ulcerated area of the skin assumed greyish color (necrotic) on the third day. The animal was perfectly active until 12 days after injection, when it showed progressive emaciation, while locally there was a solid infiltrated scar. Death occurred with symptoms of marasmus.
0.005	+ 4^h	Three hours later the animal showed grave general symptoms and collapsed. Locally there was intense swelling of the tissues without any actual ulceration.
0.005	+ $3^h 35^m$	Two hours after the injection the animal became seriously ill. Marked hæmorrhagic œdema around the site of injection, which finally spread over the entire injected side.
0.0075	§	The animal showed only slight general symptoms, while an extensive ulcer formed about the point of injection on the second day, but became dry after 7 days.
0.01	§	Showed slight stupefaction during the first day; on the second day an ulcer (3 x 4 c.c.) formed around the site of injection, but dried after 7 days.
0.015	+ $1^h 25^m$	One hour after injection the animal fell; the local swelling was very marked. At autopsy there was very marked hæmorrhage in the muscular layers, but only moderate hæmorrhage in the mesentery.

+ = Death. § = Recovery.

TABLE XI.

TOXICITY OF CROTALUS VENOM FOR RATS (200 GRM.) AFTER INTRAPERITONEAL INJECTION.

Venom in grm.	Result.
0.0002	Well.
0.0005	Well.
0.001	Well.
0.0015	Abdominal tension increased; next day well.
0.0016	Well.
0.0018	Well.
0.002	Well.
0.002	Well.
0.002	Death 4^h.
0.0022	Well.
0.003	Well.

TABLE XII.

DETERMINATION OF THE MINIMAL LETHAL DOSE OF CROTALUS VENOM (NOT FILTERED).

Guinea-pigs weighing 270 grm. were injected intraperitoneally, April 6 to 16, 1904.

Venom in grm.	Result.	Remarks.
0.00035	§	Sick for 2 days; local infiltration disappeared after 7 days.
0.0004	§	Very sick for 2 days; local infiltration marked. 9 days later the local infiltration disappeared; after 11 days the animal was quite normal.
0.00045	+ 13 days	First day, critically ill; second day, some improvement in condition, but the whole abdominal wall was softened and formed an ulcer, 3 x 3 cm., from which a sero-sanguineous fluid discharged; third day, the surface of the ulcerated area was becoming dry and the appetite was usual; fourth day, the ulcer presented a darkish hue and the edge was demarkated. After a week the ulcer dried with a blackish crust, which gradually became smaller and fell off. The animal was nevertheless much emaciated and finally died.
0.0005	+ 17^h	Animal immediately became highly irritable and uneasy after 15 to 30 minutes; abdominal tension steadily increased. None of animals showed appetite after the injection and the hairs were ruffled. In one hour the abdominal tension became very high, and the wall became a purplish color, due to hæmorrhage. A sero-sanguineous fluid was discharged from the needle puncture. The animals became weaker gradually and finally succumbed. At autopsy there was very marked hæmorrhage in the peritoneum and viscera, and in the muscular layers of the abdominal wall.
0.00055	+ 17^h	
0.0006	+ 15^h	
0.0006	+ 21^h	
0.0006	+ 19^h	
0.001	+ $3^h\,48^m$	
0.001	+ $4^h\,34^m$	

+ = Death.
§ = Recovery.

TABLE XIII.

CROTALUS VENOM AND ANTIVENIN (I) INJECTED INTRAPERITONEALLY.

Guinea-pigs weighing 250 grm. were used. April 15, 1904. The venom was

Venom 0.06 gr. + *n* c.c. Serum.	Divided by.	Result.	Symptoms of the Animals which Finally Recovered.
0.25	8	+ 10^h	
	9	+ 15^h	
	10	§	Sick for 2 days; slight infiltration around the site of injection, lasting for 5 days.
0.5	6	+ 10^h	
	7	+ 15^h	
	8	§	Slightly ill for 1 day; slight infiltration around the needle puncture lasting for 4 days.
0.75	4	+ 12^h	
	5	+ 23^h	
	6	§	Slightly ill for 1 day.
1.0	3	+ 10^h	
	3½	+ 17^h 20^m	
	4	§	Slightly ill for 1 day; slight infiltration lasting for 2 days.
	5	§	Almost no symptoms.
	6	§	Ill for several hours only.
1.25	2½	+ 15^h	Sick for 2 days; slight local infiltration lasting for 2 days.
	3	§	
1.5	1½	+ 7^h	Sick for 3 days; was very ill the first day. Local infiltration lasted for 3 days.
	2	§	
1.75	1	+ 7^h	
	1½	+ 15^h	Sick for 3 days; marked local swelling, lasting for 3 days.
	2	§	
2.0	1	+ 15^h	
	1½	§	Sick for 2 days; local infiltration for 3 days.
2.25	½	+ 17^h	
	½	+ 12^h	Very sick for 2 days; local infiltration marked and lasted for 3 days; recovery in 4 days.
	1	§	
2.5	½	§	Slightly ill for one day; recovery in 2 days.
	1	§	Almost no symptoms.
	1	§	No symptoms.
	1	§	No symptoms.

+ = Death.
§ = Recovery.

TABLE XIV.

CROTALUS VENOM-ANTIVENIN INJECTED INTO GUINEA-PIGS.

n	q Obs.	q Calc. I.	q Calc. II.
0	12	11.5	11.8
0.25	9	9	8.7
0.5	7	7	6.4
0.75	4.5	5.2	4.7
1	3.5	3.7	3.5
1.25	2.5	2.5	2.55
1.5	1.7	1.8	1.9
1.75	1.5	1.25	1.4
2	1	0.87	1.1
2.25	0.5	0.5	0.55
$p = 1$.	$K = 0.048$.	$K = 0.053$.	$n = 3/2$.

TABLE XV.

TOXICITY OF CROTALUS VENOM UPON RABBIT.

1. *Subcutaneous Injection.* (*Left Inguinal Region.*)

Venom in grm.		Weight of Animal.	Result.
0.01	25/iv	2000 grm.	+ 7 days
0.015	26/iv	2100	+ 18^h
0.02	27/iv	2100	+ 10^h 25^m
0.02	16/v	1600	+ 7^h 40^m
0.04	17/v	1700	+ 2^h 40^m

2. *Intravenous Injection.*

0.00025	7/vi	2000 grm.	§
0.0003	7/vi	2000	+ 9 days
0.0003	2/vi	1800	+ 3 days 12^h
0.0003	24/vi	1450	+ 3 days 4^h
0.0004	30/vi	1550	§
0.0004	7/vi	2000	+ 4 days 16^h
0.0004	15/vi	2000	§
0.0005	15/vi	2000	+ 4 days 16^h
0.0005	7/vi	2000	+ 3 days
0.0005	2/vi	1750	+ 17^h 50^m
0.0005	20/v	1600	+ 14^h
0.001	20/v	1600	+ 6–14^h
0.000461	11/vii	1650	§
0.0005	11/vii	1650	+ 5 days.
0.000545	11/vii	1550	+ 4 days 20^h
0.0006	11/vii	1500	+ 4 days

+ = Death.
§ = Recovery.

TABLE XVI.

CROTALUS VENOM-ANTIVENIN INJECTED INTRAVENOUSLY.

Rabbits weighing about 1700 grm. were used. July 10 to 20, 1904. The venom was not filtered. Crotalus Antivenin I was used.

Venom 0.006 grm. + *n* c.c. Serum.	Divided by.	Result.
0	10	+ 4 days 12h
	11	+ 4 days 20h
	12	+ 5 days
	13	§
0.5	4	+ 4 days 12h
	5	+ 5 days 7h
	6	§
	7	§
1.0	2	+ 5 days 7h
	3	+ 5 days 9h
	4	§
1.5	1½	+ 5 days 6h
	2	+ 5 days 9h
	2½	§
2.0	1½	+ 4 days 12h
	2	§
	2½	§
2.5	½	+ 4 days 12h
	1	+ 5 days 20h
	1½	§
3	1	§

+ = Death. § = Recovery.

TABLE XVII.

CROTALUS VENOM-ANTIVENIN.

n	*q* Obs.
0	12
0.5	5
1.0	3
1.5	2
2.0	1.5
2.5	1.0

TABLE XVIII.

CROTALOLYSIN.

5 per cent. suspension of dog's blood.
1 c.c. of 0.05 per cent. crotalus venom.
+ *n* c.c. of anticrotalus serum.
+ 1 — *n* c.c. 0.9 per cent sodium chloride solution.

n	I.	II	III.	*q* Obs.	*q* Calc.
0	0.02	0.016	0.006	100	100
0.05	0.023	0.018	0.0065	89	90.5
0.1	0.026	0.02	0.0078	77.4	81
0.15	0.028	0.023	0.0085	70.6	72

x	I.	II.	III.	q Obs.	q Calc.
0.2	0.03	0.025	0.01	63.5	62.5
0.25	0.033	0.029	0.0125	54.4	53
0.3	0.0385	0.035	0.015	45.9	44
0.35	0.052	0.043	0.019	35.8	34.5
0.4	0.078	0.065	0.026	24.4	25
0.45	0.11	0.098	0.047	15.8	16
0.5	0.275	0.24	0.12	6.3	6.5
0.55	1.5	1.15	0.35	1.5	0

TABLE XIX.

DETERMINATION OF THE MINIMAL LETHAL DOSE OF COBRA VENOM.

Stock solution was made as follows: Dried venom, 0.1 grm.; distilled water, 50 c.c.; no filtration. Guinea-pigs weighing 650 grm. were injected subcutaneously February 15, 1904.

Venom in grm.	Result.
0.00005	§
0.0001	+ 23 days
0.00015	§
0.0002	+ 36^h
0.00025	+ $18^h\ 29^m$
0.0003	+ 12^h
0.0005	+ $3^h\ 35^m$
0.00075	+ $2^h\ 3^m$
0.001	+ $3^h\ 34^m$
0.0015	+ $1^h\ 30^m$

+ = Death. § = Recovery.

TABLE XX.

DETERMINATION OF THE MINIMAL LETHAL DOSE OF COBRA VENOM.

Stock solution: Dried venom, 0.4 grm.; distilled water, 100 c.c.; filtered through a Chamberland bougie. Guinea-pigs were injected subcutaneously February 20 to March 2, 1904.

Venom in grm.	Weight of Animals in grm.	Result.
0.0001	530	§
0.00015	470	§
0.0002	550	§
0.0002	480	§
0.00025	450	§
0.0003	460	+ 13^h
0.0004	450	+ $4^h\ 39^m$
0.0005	450	+ $2^h\ 35^m$
0.001	460	+ $1^h\ 59^m$
0.0015	620	+ $1^h\ 42^m$
0.002	600	+ $1^h\ 43^m$

+ = Death. § = Recovery.

TABLE XXI.

RELATION BETWEEN DOSE AND TOXICITY.

Dose in grm.	M. l. d.	Time of Death in Hours.
0.0002	0.4	36
0.00025	0.5	18.5
* "	"	17
0.0003	0.6	12
* 0.0004	0.8	6.16
0.0005	1	3.5
* "	"	4
0.00075	1.5	2
0.001	2	3.5
* "	"	3.67
0.0015	3	1.5
* "	"	1.75
* 0.002	4	1.75

TABLE XXII.

DETERMINATION OF THE MINIMAL LETHAL DOSE OF COBRA VENOM WHEN INJECTED WITH LECITHIN.

Stock solution of cobra venom was made as follows: Dried venom, 0.4 grm.; distilled water 100 c.c.; filtered through Chamberland bougie.

The dose of lecithin injected with the venom was 1 c.c. of 1/50 N. solution. The animals used were guinea-pigs weighing 600 gram. The venom was injected subcutaneously February 24, 1904.

Venom in grm.	Result.
0.0002	§
0.0003	+ 7^h
0.0004	+ $10^h\ 30^m$
0.0005	+ $3^h\ 47^m$

+ = Death.
§ = Recovery.

TABLE XXIII.

VELOCITY OF THE REACTION BETWEEN COBRA VENOM AND CALMETTE'S ANTIVENIN.

The venom-antivenin mixture was injected intraperitoneally into guinea-pigs weighing 600 grm.

Cobra venom 0.008 grm. + Calmette's antivenin 5 c.c.

Simultaneously	1 Hour Contact at 36° C.	6 Hour Contact at 36° C.
+ 90 minutes	+ 85 minutes	+ 85 minutes

TABLE XXIV.

Cobra Venom and Calmette's Antivenin I.

Calmette's Antivenin I. 17/ii/'04.				Calmette's Antivenin I. 17/ii/'04.			
Weight of Guinea Pig in grm.	Venom 0.008 grm. + n c.c. Serum.	Divided by	Result.	Weight of Guinea Pig in grm.	Venom 0.004 grm. + n c.c. Serum.	Divided by	Remarks.
650	3	4	+ 8 min.	710	3.0	10	+ $3^h\ 4^m$
650		7	+ $3^h\ 12^m$	670		$13\frac{1}{2}$	+ $10^h\ 20^m$
650		10	+ $2^h\ 8^m$	690		20	+ $15^h\ 20^m$
				770		28	+ $5^h\ 25^m$
700	4	4	+ $3^h\ 2^m$				
700		5	+ $3^h\ 33^m$				
690		6	+ $3^h\ 36^m$				
550		4	+ $2^h\ 35^m$				
540	5	6	+ $2^h\ 50^m$				
550		10	+ $7^h\ 50^m$				
590		$13\frac{1}{2}$	§				

+ = Death.
§ = Recovery.

TABLE XXV.

Cobra Venom and Calmette's Antivenin II.

The venom solution was filtered through the Chamberland bougie before use March 9, 1904.

Calmette's Antivenin II.				
Quantity of the Antivenin Used in c.c.	Cobra Venom in grm.	Weight of Guinea Pig in grm.	Result.	Remarks.
10	0.004	450	+ 25^m	
10	0.0036	430	+ 43^m	
10	0.0032	480	+ $5^h\ 41^m$	
10	0.0028	450	§	No general symptoms; a slight local œdema lasting for 2 days.
10	0.002	440	§	No symptoms.
One bottle (ca. 12).	0.008	510	+ 26^m	
"	0.0048	480	+ 32^m	
"	0.004	570	§	No general symptoms; marked local œdema lasting for 4 days; no loss in weight.
"	0.0032	490	§	No symptom.
Normal horse serum, 20	0.008	600	+ 15^m	
Normal horse serum, 20		600	§	Slightly inactive after 1 hour, but well after 6 hours.

+ = Death.
§ = Recovery.

TABLE XXVI.

Cobra Venom and Calmette's Antivenin. II.

The venom solution was filtered before use. The mixture of venom and antivenin was injected intraperitoneally into guinea-pigs.

Series A. Guinea Pigs of 370 grms.				Series B. Guinea Pigs of 450 grms.			
Calmette's Antivenin II. 11-12/iii/'04				Calmette's Antivenin II. 11-12/iii/'04.			
Venom 0.0028 grm. + *n* c.c. Serum.	Divided by.	Result.	Remarks.	Venom 0.0028 grm. + *n* c.c. Serum.	Divided by.	Result.	Remarks.
2	5	+ 12h		2	4	+ 27m	
	6	+ 26h			4	+ 17h 40m	
	7	§	Slightly ill for one day.		5	+ 48h	
	8	§	Almost no symptoms.		6	§	Almost no symptoms.
4	3	+ 55m		4	2.5	+ 1h 36m	
	4	+ 36h			3	+ 14h 40m	
	5	§	Sick for one day.		4	§	Sick several hours.
	6	§	Almost no symptoms.	6	2	+ 40h	
6	2.5	+ 60m			2.5	§	Sick for two days.
	3	+ 26h 9m			3	§	Slightly ill for several hours.
	4	§	Slightly ill for one day.	8	1	+ 31m	
8	1.5	+ 28m			1.5	+ 1h 6m	
	2	§	Slightly inactive for several hours.		2	§	Sick for two days.
	2.5	§	No symptoms.		2.5	§	Slightly ill for one day.
9	1	§	Sick for one day.	10	1	§	Almost no symptoms.
10	1	§	No symptom.				

+ = Death.
§ = Recovery.

TABLE XXVII.

NEUTRALIZATION OF COBRA VENOM WITH CALMETTE'S ANTIVENIN.

The venom-antivenin mixture was injected subcutaneously into guinea-pigs weighing 250 grm.

Cobra Venom 0.003 grm. +*n* c.c. Serum.	Divided by.	Result.
0	8	+ 2^h
	9	+ $3^h\ 30^m$
	10	+ $2^h\ 30^m$
	11	+ 3^h
	12	+ 36^h
	13	§
	14	§
	15	§
1.0	5	+ 4^h
	6	+ 6^h
	7	+ 8^h
	7	+ $2^h\ 30^m$
	8	§
	8	§
2.0	3	+ 5^h
	4	+ $2^h\ 30^m$
	4	+ 4^h
	5	§
	5	§
	6	§
3.0	1	+ 36^h
	2	+ 3 days
	2	§
	2.5	§
3.5	1	+ $5^h\ 20^m$
	1	+ $3^h\ 45^m$
	1.5	§
4	1	§
	1	§

+ = Death. § = Recovery.

TABLE XXVIII.

1 c.c. 0.1 % Cobra Venom. + *n* c.c. of Antivenin I. + 1−*n* c.c. of Salt Solution (0.9 %).			1 c.c. 0.1 % Cobra Venom. + *n* c.c. of Antivenin II. + 1−*n* c.c. of Salt Solution (0.9 %).		
n	*q* obs.	*q* calc.	*n*	*q* obs.	*q* calc.
0	100	100	0	100	100
0.1	78.2	79.5	0.1	83.5	82.5
0.2	56.6	59.5	0.15	71.3	74
0.3	39.8	40.5	0.2	64.5	66
0.4	21.6	20.5	0.25	56.2	57
0.5	5.3	1	0.3	44.4	48.5
			0.4	33.3	32
			0.5	15.9	15
			0.6	3.3	0

TABLE XXIX.

DETERMINATION OF THE MINIMAL LETHAL DOSE OF WATER MOCCASIN VENOM.

Stock solution was made as follows: Dried venom, 1.0 grm.; distilled water, 100 c.c.; the solution was not filtered. The venom was injected intraperitoneally into guinea-pigs May 5, 1904.

Venom in grm.	Weight of Animal in grm.	Result.	Remarks.
0.0005	250	§	Sick for one day.
0.0008	250	§	Ditto.
0.001	250	§	Ditto.
0.001	250	§	Ditto.
0.001	250	§	Ditto.
0.0012	250	+ 7^h	Marked hæmorrhage in the abdominal cavity.
0.0012	250	+ $6^h\ 20^m$	Ditto.
0.0014	250	+ 45^m	Very marked hæmorrhage in the abdominal cavity.
0.0024	420	+ $4^h\ 40^m$	Ditto.
0.0024	420	+ $4^h\ 35^m$	Ditto.
0.0024	420	+ $3^h\ 50^m$	Ditto.
0.0024	420	+ $4^h\ 20^m$	Ditto.

+ = Death.
§ = Recovery.

TABLE XXX.

DETERMINATION OF THE MINIMAL LETHAL DOSE OF WATER MOCCASIN VENOM.

The stock solution of venom was as follows: Dried venom, 1 grm.; distilled water, 100 c.c. The solution which was not filtered was preserved in the ice chest for 108 days. Animals were injected August 22, 1904.

Venom in grm.	Weight of Animal in grm.	Result.
0.0006	250	§
0.0008	250	§
0.001	250	§
0.001	250	§
0.0012	250	+ 5^h
0.0012	250	+ 6 days
0.0012	250	+ $5^h\ 20^m$
0.0013	250	+ 5^h
0.0014	250	+ 4^h
0.0014	250	+ 4^h
0.0024	420	+ $4^h\ 40^m$
0.0024	420	+ $4^h\ 35^m$
0.0024	420	+ $3^h\ 50^m$
0.0024	420	+ $4^h\ 20^m$

+ = Death.
§ = Recovery.

TABLE XXXI.

Neutralization of Water Moccasin Venom.

The time of contact was 3 hours at 37° C. The animals used were guinea-pigs weighing 250 grm., and the injection was made intraperitoneally.

Venom 0.012 grm. + *n* c.c. of Serum.	Divided by.	Result.
2	5	+ 9h
	5	+ 6h
	6	+ 6h 30m
	6	+ 10h 17m
	7	§
	7	§
	8	§
	8	§
4	3	+ 3 days
	4	+ 5h 30m
	4	+ 16h
	5	+ 6h 20m
	5	§
	6	§
5	3	+ 7h 20m
	4	§
	5	§
6	2	+ 5h
	2.5	+ 16h and 5h
	3	§
	3	§
	4	§
8	1.5	+ 6h 30m
	2	+ 18h
	2	+ 16h
	2.5	+ 22h
	2.5	§
9	1	+ 3h 10m
	2	+ 4h 10m
	3	+ 14h
10	1.5	+ 5h 30m
	2	§
	2.5	+ 6h 40m
	3	+ 7h 10m
20	2	+ 9h
	3	+ 3h
	4	+ 3h
40	4	+ 3h

TABLE XXXII.

1 c.c. of 0.05 per cent. solution of Ancistrodon piscivorus venom + 2 c.c. of antivenin + 1 — n c.c. of 0.9 per cent sodium chloride solution.

n	q Obs.	q Calc.
0	100	100
0.05	93	94
0.1	87	88
0.15	82	82
0.2	77	76
0.25	70	70
0.3	63	64
0.4	53	52
0.5	42	40
0.6	26	28
0.7	17	16
0.8	10.5	4
1	2	0