THE ACTION OF COBRA POISON ON THE BLOOD: A CONTRIBUTION TO THE STUDY OF PASSIVE IMMUNITY.[1]

By J. W. W. Stephens, M.B., *John Lucas Walker Student in the University of Cambridge*; and W. Myers, M.A., M.B.

From the Pathological Laboratory of the University of Cambridge.

Part I.

The exact action taking place between a toxin and an antitoxin is still unknown, and more or less a matter of conjecture. Some observers, as, for instance, Roux and Metchnikoff, believe that an antitoxin can neutralise a toxin only in the presence of living cells, *i.e. in corpore*, and that *in vitro* it does not affect the toxin. Others, and notably Ehrlich, believe that when toxin and antitoxin come together, a chemical reaction takes place in the test-tube as well as in the body. Buchner (1) mixed tetanus toxin and tetanus antitoxin together in test-tubes in such a manner that the resulting mixture was harmless to mice, or contained even an excess of antitoxin for mice. Nevertheless he found that this mixture was still toxic for guinea-pigs. Hence he argues that in the test-tube the antitoxin does not destroy the poison. But, as Behring (2) pointed out, Buchner's experiments are not convincing, because guinea-pigs are more susceptible to tetanus toxin than mice; and Kanthack (3) expresses himself in the same sense when he says: "We must not forget that there is no absolute standard of virulence or toxic effect; that the toxic effect must naturally vary for each animal; thus, in estimating whether a poison has been rendered innocuous, we must use the most susceptible animals. We shall then find that a toxin, to which serum has been added so as to neutralise it for such animals, will be harmless for all others. A more or less refractory animal is, from its very nature, able to account for a greater or less fraction of the poison, so that the protective serum is only called upon to neutralise the surplus. This may explain Buchner's difficulty, that a mixture of tetanus toxin and antitetanic serum, though harmless to the more refractory mouse, is still harmful to the more susceptible guinea-pig."

[1] Communicated to the Pathological Society, 1st March 1898.

Roux and Calmette ([4]), on the other hand, have shown that if a mixture of snake venom and its antitoxin, in such proportions as to be harmless to animals, be heated to 68° C. for ten minutes, the toxic action of the snake venom reasserted itself; so that it is evident, according to them, that the poison actually existed as such in the mixture. Wassermann ([5]), working with pyocyaneus toxin, similarly comes to the conclusion that in a mixture of pyocyaneus toxin and pyocyaneus antitoxin, in such proportions as to be neutral for guinea-pigs, the poison remains intact, and is destroyed only after the mixture has been injected into the tissues; so that the antitoxin does not directly act upon the toxin, but only in the presence of living tissues, which liberate from the antitoxin the substances capable of neutralising the toxin.

It must be obvious that the experiments of Roux, Calmette, and Wassermann are as little convincing as are those of Buchner. For toxin and antitoxin may react upon each other chemically *in vitro*, and form a physiologically neutral compound, and yet this new compound may be at once dissociated by heat, which would further destroy the antitoxin thus split off. It would not be difficult to adduce examples from chemistry to give support to our objections.

In 1896 Professor Kanthack demonstrated before the Physiological Society at St. Bartholomew's Hospital a few test-tube experiments, which proved that an antitoxin is capable of acting upon the corresponding toxin *in vitro* in the absence of living tissues. He started from D. D. Cunningham's ([6]) observation, that cobra poison, when mixed with shed blood in a test-tube, prevents clotting.

On adding, however, antivenomous serum to the cobra poison in proper proportions previous to mixing it with the blood, clotting takes place as quickly as it does under normal conditions.

Again, the blood of an immunised animal mixed with a certain calculated quantity of cobra poison clots as rapidly as ordinary blood. There can be no doubt, therefore, from these preliminary and unpublished observations, that the cobra antitoxin is capable of acting upon the cobra poison outside the animal body. As Behring maintains, the poison is not necessarily destroyed, it is neutralised. Kanthack further showed that this action of cobra antitoxin is specific; for normal serum, diphtheritic antitoxin, and antityphoid serum had no influence over the cobra poison *in vitro*, and clotting did not set in.

These observations cannot be explained on a mechanical basis, but we are almost forced to assume that a chemical change has taken place *in vitro* between toxin and antitoxin, and that this change is a specific one. Vital influences play no part, because the experiments were performed outside the body, and clotting is not a vital phenomenon. Here, then, we have evidence that the living organism

is not a necessary intermediary for the liberation of the active molecules from the antitoxin.

In 1897 Ehrlich (7) published some important experiments, which proved, even more completely than Professor Kanthack's unpublished ones, that the toxin reacts chemically upon the antitoxin *in vitro*. His experiments were, as might be expected, most carefully thought out. Ricin produces a curious effect upon defibrinated blood, the corpuscles being firmly clumped together and precipitated. Ehrlich filled six test-tubes, each with 95 c.c. of physiological saline solution, containing 0·5 per cent. citrate of sodium, and added thereto 5 c.c. of rabbit's blood. 1 c.c. of a 2 per cent. ricin solution quickly precipitates the red corpuscles. He now added to five of the above test-tubes severally, 1 c.c. of 2 per cent. ricin solution, mixed respectively with 0·3, 0·5, 0·75, 1·0, and 1·25 c.c. of diluted antitoxin, while to the sixth tube he added 1 c.c. of the ricin solution without the antitoxin. The result was that the addition of 1·25 and 1·0 c.c. of antiricin neutralised the action of the ricin in the test-tube, while 0·3 c.c. had no appreciable effect, and 0·5 merely delayed the clumping and precipitation, whereas with 0·75 c.c. the clumping was imperfect as well as delayed. He now proceeded to test these mixtures of ricin and antiricin upon mice, and found that the addition of 1·25 and 1·0 c.c. of antiricin had neutralised the ricin for the animal also; while 0·3 c.c. had no effect, 0·5 delayed death, and 0·75 c.c. weakened its action to such an extent that nothing more than a moderate infiltration resulted. It is evident, therefore, that the animal experiments confirm the test-tube experiments, and Ehrlich has thus shown that ricin and antiricin directly influence each other chemically without the assistance of cellular activity. Further (8), he asserts that by means of test-tube experiments it can be shown that toxin and antitoxin unite much quicker in concentrated than in dilute solutions; that warming hastens this union, and cold delays it. He reminds us that analogous phenomena are readily found in chemistry, especially in the formation of double salts, and it is therefore possible that the neutralisation of toxins by antitoxins represents the formation of a double salt.

Ehrlich's views have recently received strong support through Wassermann's (9) observations upon a new method of immunisation, which consists in saturating the body with those tissue substances for which the toxin has a great affinity. Thus tetanus toxin has a strong and almost specific affinity for certain cell groups of the central nervous system, and Wassermann has shown that by mixing an emulsion of cord or brain with the tetanus toxin, either *in vitro* or *in corpore*, it is possible to bind and saturate the toxophoric atom groups of the toxin before they reach the nerve-cells of the animal. These experiments tend to show that the neutralisation of a toxin which possesses toxophoric atom groups having a strong affinity for certain

tissue substances, may be effected by saturating or binding these atom groups with those substances, *i.e.* by chemical combination. This is effected inside the body as well as outside, and therefore we have some excuse for inclining towards Ehrlich's chemical reasoning rather than towards Roux's more vitalistic hypothesis.

It is evidently important, if we wish to understand the remarkable actions of antitoxins, to search, like Kanthack and Ehrlich have done, for simple reactions which can be demonstrated outside the body in test-tubes. Following, therefore, a suggestion of Professor Kanthack, to whom we are indebted for valuable advice, we determined to study by means of test-tube reactions—(1) the action of cobra poison upon blood; (2) the effect of Calmette's antitoxin upon this action, *i.e.* whether the antitoxin would, as maintained by Professor Kanthack, neutralise the poison *in vitro*; (3) it remained to be seen whether the neutralising point *in vitro* was also the absolutely neutral point *in corpore* for the animal whose blood was used in the experiment.

THE ACTION OF COBRA POISON UPON THE BLOOD *in Vitro*.

When cobra poison is added to shed blood in a test-tube, two effects are noticeable—(*a*) hæmolysis, meaning thereby destruction of the red corpuscles, and laking of the blood; and (*b*) delay or complete absence of clotting. In the following experiments we have chosen hæmolysis as our test reaction.

PART I. HÆMOLYTIC ACTION OF COBRA POISON AND THE INFLUENCE OF ANTIVENOMOUS SERUM UPON THIS PHENOMENON *in Vitro*.

The Hæmolytic Property of Cobra Poison.

It is well known that a solution of cobra poison has a definite action on the red corpuscle, one result of which is to liberate the hæmoglobin, so that the solution becomes laky. These observations have generally been made by mixing a little of the poison solution with a little saline solution under a cover glass, and by subsequent examination under the microscope. The corpuscles swell, losing their biconcave form; then becoming more and more shadowy, till eventually they disappear. This method is not a convenient one, as it requires prolonged observation of a specimen under the microscope, besides, and at best it is only a rough method, as it does not give us an accurate measure of the hæmolysis, since we do not know exactly what quantities of poison solution and blood are being employed. A more accurate method is to mix the blood and poison solutions in a hæmacytometer pipette, and then to count a given field, and from time to time to observe whether there is any change in the cells or any decrease

in their number. Besides the inconvenience of keeping the same field under observation for many hours, the fact that the onset of any change at all is under certain conditions much delayed, may lead to the error of recording a really positive action of the poison as negative. The advantage of this method is that it gives us a numerical estimate of the activity of the poison. Thus, using a mixture of blood and cobra poison of varying strengths in the proportion of 1 of human blood to 200 of the poison solutions, we got the following results :—

TABLE I.—*Cobra Poison in* ·5 *per cent. Saline* (1 c.c. = ·2 mgrms. of poison).

5. 5	P.M.,	58	red cells in	16 squares.
5.10	,,	17	,,	,,
5.12	,,	12	,,	,,
5.15	,,	1	,,	,,

TABLE II.—*Cobra Poison in* ·5 *per cent. Saline* (1 c.c. = ·1 mgrm. of poison).

11.40	A.M.,	97	red cells in	16 squares.
11.45	,,	72	,,	,,
12. 0	,,	57	,,	,,
12.10	P.M.,	31	,,	,,
12.20	,,	26	,,	,,

TABLE III.—*Cobra Poison in* ·5 *per cent. Saline* (1 c.c. = ·05 mgrm. of poison).

10.45	A.M.,	132	red cells in	16 squares.
11.32	,,	99	,,	,,

The last example shows that the action is slow with dilute poison solutions,—further on we shall show that the result may be even longer delayed.

The initial number of red cells in these examples—58, 97, and 132—also gives us some idea of the relative activity of the solutions of different strength; and further, the rate of diminution shows us the same: thus, in Table 1 all the red cells had disappeared in ten minutes, whereas in Table 3 a diminution of thirty-three only had occurred in forty-seven minutes.

The observation of the effects of small quantities of the poison we think can be most satisfactorily conducted by making the mixtures in small test-glasses. The mixtures are allowed to stand for some hours—generally twelve hours—and it is then observed whether the fluid above the corpuscles is tinged with hæmoglobin or not: in doubtful cases we employed the spectroscope.

Keeping in mind, however, the fact fully elaborated by Hamburger[10], that blood is laked by distilled water and saline solution, which are hypotonic with regard to the red corpuscles, it is necessary, moreover, that the poison should be dissolved in a solution, which is isotonic or hypertonic for the particular blood under observation, in order that the effect of the poison alone may be estimated.

The following table gives the equivalent isotonic solutions for the various samples of bloods tested :—

TABLE IV.

Animal.	Salt per cent.	Animal.	Salt per cent.
Rabbit . . .	·5 –·6	Toad (winter) . .	·3
Guinea pig . .	·45–·5	Rabbit . . .	·5–·6
Dog . . .	·45–·5	Cat . . .	·5–·6
Man . . .	·45–·5	Snake (winter) .	·4
Fowl . . .	·3 –·4	Rat . . .	·4–·5
Frog (winter) . .	·1 –·2		

Hence, for instance, in comparing the action of poison solutions on frog's blood and human blood, the solutions must be made respectively in ·2 per cent. saline and in ·5 per cent. saline.

Nor must the solutions be strongly hypertonic, for, with poison solutions of a certain strength, no hæmolysis may be obtained if a 1 per cent. salt solution be employed, whereas in an isotonic solution hæmolysis is active. Thus, using 1 per cent. saline as the diluent, the following results on the different bloods were observed :—

TABLE V.

Poison actually Present.	Dog.	Guinea-pig.	Frog.	Man.
·5 c.c. = ·02 mgrms. . .	Complete H.	H. incomplete.	Complete H.	No. H.
,, = ·01 ,, . .	Much H. (deposit).	Trace.	No H.	,,
,, = ·005 ,, . .	,,	No H.	,,	,,
,, = ·0025 ,. . .	H. (deposit).	,,	,,	,,
,, = ·00125 ,, . .	H. slight.	,,	,,	,,
,, = Saline 1 per cent. .	No H.	,,	,,	,,

Note.—To each tube a standard platinum loop full of blood is added and shaken up, and after standing twelve hours the observations are recorded.

H. = hæmolysis. "Complete" signifies that after standing twelve hours there was no sediment at the bottom of the tube. "Trace" generally implies that a spectroscope was necessary to detect the hæmoglobin in solution.

Thus we see that a 1 per cent. saline solution containing ·02 mgrm. of poison does not hæmolyse man's blood, while if a ·5 per cent. solution be used, complete hæmolysis ensues (*vide* Table IX.).

This result, however, must only be considered to hold good for the particular strengths of poison and saline of this experiment, for

with stronger poison solution hæmolysis will proceed in solutions of salt containing as much as 10 per cent., as the following table shows:—

TABLE VI.

Cobra Poison 1 c.c. = ·2 mgrms.		Series I.	Series II.
Poison actually present.	·5 c.c. = ·1 mgrm. + ·5 c.c. 20 per cent. saline.	...	H. complete.
	,, = ,, + ,, 10 ,,	H. incomplete.	,,
	,, = ,, + ,, 5 ,,	No H.	,,
	,, = ,, + ,, 2·5 ,,	H. incomplete.	,,
	,, = ,, + ,, 1·25 ,,	H. complete.	,,
	,, = ,, + ,, ·625 ,,	,,	,,

Taking these observations together with those recorded in Table V., they show the necessity for using isotonic solutions, or at least stating in any experiment the exact strength of the salt solution.

It is interesting in this connection to consider what the action of strong salt solutions alone is upon blood, thus:—

TABLE VII.

	Series I.	Series II.
·5 c.c. 20 per cent. saline	...	H. Slight.
,, 10 ,, ...	Slight H. in 24 hours.	,,
,, 5 ,, ...	No H.	H. trace.
,, 2·5 ,, ...	,,	No H.
,, 1·25 ,, ...	,,	,,
,, ·625 ,, ...	,,	,,

That these results cannot be attributed to bacterial action is shown by the fact of their occurring only in solutions of definite strength, the remaining tubes showing no such change. If, however, the tubes be allowed to stand from twenty-four to forty-eight hours without taking strict aseptic precautions, they all show hæmolysis, which, however, is generally slight ([11]). As a rule, we did not consider it necessary to take more than ordinary precautions, which were indeed sufficient, as shown by the fact that our control saline tubes, without any poison, in twelve hours never showed any hæmolysis.

Another peculiar fact we have established is, that poison solutions, containing from 2 mgrms. to 7·5 mgrms. in 1 c.c., hæmolyse blood,

occasionally not at all; at other times, less completely than weaker solutions do. Thus—

TABLE VIII.

Poison Actually Present.	Series I.	Series II.
·5 c.c. = 7·5 mgrms.	No H. deposit.	H. incomplete.
,, = 3·75 ,, ...	,,	,,
,, = 1·87 ,, ...	,,	,,
,, = ·93 ,, ...	H. complete.	H. complete.
,, = ·46 ,, ...	,,	
,, = ·23 ,, ...	,,	
,, = Saline, ·5 per cent., . . .	No H.	

Solutions of a greater strength than 1 c.c. = 15 mgrms. we have not employed, and we do not propose here to discuss the reason of this phenomenon, but reserve the question for a future communication.

We found in our earlier experiments that the blood of different animals behaved differently with respect to this hæmolytic property of cobra poison, and the results given in Table IX., where for each blood its corresponding isotonic solution was used, were obtained for different kinds of blood.

While, therefore, the isotonic point for saline solutions is identical for rat, snake, fowl, man, and guinea-pig, the isotonic point, if we may use the expression, for cobra poison, is very different, as is seen by the table. Dog's blood is exceedingly sensitive to the poison; hæmolysis in this animal occurring in very dilute solution, for instance, in the strength ·5 c.c. = ·0009 mrgm.

Further, with regard to dog's blood (and the same holds for the frog), it was observed that the hæmolysis was often complete in less than one hour in the solutions of various strengths, whilst in the corresponding tubes for guinea-pig and man, hæmolysis was not apparent for three to four hours, though eventually complete.

We should point out that these numbers must not be taken as absolute, as from time to time variations occur in the minimum amounts of poison required to produce hæmolysis. These, however, are not sufficiently large to negative the general relation shown above, and may be due to changes in the poison, or the blood, or in both.

The Action of Antivenomous Serum on the Hæmolytic Property of Cobra Poison.[1]

This antivenomous serum itself is hypertonic for human and

[1] The serum used in these experiments was that prepared at the Pasteur Institute, Lille, by Dr. A. Calmette.

TABLE IX.

Poison Present.	Rabbit, ·6 per cent. Saline.	White Rat, ·5 per cent. Saline.	*Tropidonotus natrix*, ·5 per cent. Saline.	Man, ·5 per cent. Saline.	Toad, ·3 per cent. Saline.	Cat, ·6 per cent. Saline.	Frog, ·3 per cent. Saline.	Fowl, ·5 per cent. Saline.	Guinea-Pig, ·5 per cent. Saline.	Dog, ·6 per cent. Saline.
·5 c.c. = ·5 mgrm.	H.	Complete H.	C. H.	C. H.	C. H.	H.	H.	H.	C. H.	H.
,, = ·25 ,,	Slight H.	H.	,,	,,	H.	,,	,,	,,	H.	,,
,, = ·125 ,,	Trace H.	,,	,,	,,	,,	,,	,,	,,	,,	,,
,, = ·0625 ,,	No H.	Slight H.	,,	,,	,,	,,	,,	,,	,,	,,
,, = ·031 ,,	,,	No H.	Slight H.	,,	,,	,,	,,	,,	,,	,,
,, = ·015 ,,	,,	,,	Trace H.	C. H.	H.	,,	,,	,,	,,	,,
,, = ·007 ,	,,	,,	No H.	Trace H.	No H.	,,	,,	Slight H.	,,	,,
,, = ·0039 ,,	,,	,,	,,	No H.	,,	H.	,,	,,	Slight H.	,
,, = ·0019 ,,	,,	,,	,,	,,	,,	Slight H.	H.	Slight H.	Slight H.	,,
,, = ·0009 ,,	,,	,,	,,	,,	,,	No H.	Trace H.	No H.	Trace H.	,,
Saline	,,	,,	,,	,,	,,	,,	No H.	,,	No H.	No H.

Note.—The double line marks the point where the H. was well marked. The single line marks the point where the H. was very slight.

guinea-pig's blood; and the isotonic point is shown by the following table:—

TABLE X.

·6	Antivenomous serum	+ ·4 distilled water .	No H.
·55	,,	+ ·45 ,, .	,,
·50	,,	+ ·5 ,, .	H incomplete.
·4	,,	+ ·6 ,, .	H complete.

Consequently we have used in the experiments a mixture of serum and water in the proportion of 55 : 45.

If, however, blood be added to undiluted serum, the corpuscles quickly collect at the bottom of the tube and on the sides in little granular masses, which have a peculiar brick-red colour, differing from that of a deposit of corpuscles in hypertonic saline. These masses, if examined under the microscope, are seen to consist of isolated clumps, the cells in which are much distorted and elongated, and are closely adherent to one another, the appearance suggesting a kind of agglutination of the corpuscles, a phenomenon due no doubt to the fact that we are using the serum of a horse. If water be added in increasing quantities, this appearance becomes less marked, until below the isotonic point the corpuscles swell, and are finally dissolved. The clumps can be seen under the microscope to break up, and the individual corpuscles to become detached.

We now proceeded to take solutions of poison which readily hæmolyse, and to try what the action of serum was on this phenomenon, and we found that the action could be completely arrested by using definite quantities of serum.

TABLE XI.—*Cobra Poison*, 1 c.c. = ·2 mgrm., *in Isotonic Salt Solution—Human Blood.*

·5 c.c. C. P.[1]	. . . . .	.	Complete H. (in 1 hour).
,, ,,	+ ·001 c.c. isotonic serum	.	Complete H.
,, ,,	+ ·025 ,, ,,	.	Incomplete H.
,, ,,	+ ·05 ,, ,,	.	,,
,, ,,	+ ·1 ,, ,,	.	No H.
,, ,,	+ ·2 ,, ,,	.	No H. Much clumping.
,, ,,	+ ·3 ,, ,,	.	,, ,,
,, ,,	+ ·4 ,, ,,	.	,, ,,
,, ,,	+ ·5 ,, ,,	.	,, ,,

The same results were obtained by varying the poison and keeping the serum constant.

Repeated observations showed that 1 c.c. of the particular isotonic serum used was always sufficient to stop the hæmolytic action of ·5 c.c. of a solution of poison (1 c.c. = ·2 mgrm.) on human blood.

Further, when we took multiples of these numbers, the same relation held good.

[1] C. P. is used for Cobra Poison.

TABLE XII.—*Cobra Poison*, 1 c.c. = ·2 mgrm.

·5 c.c.	C. P.	+ ·1 isotonic serum	No. H.
1 ,,	,,	+ ·2 ,, ...	,,
2 ,,	,,	+ ·4 ,, ...	,,
3 ,,	,,	+ ·6 ,, ...	,,

These observations show that the antivenomous serum possesses the power of neutralising the hæmolytic action of cobra poison *in vitro*, in the same manner as the antiricin neutralises ricin in the test-tube.

Our next experiments were directed to ascertaining whether this counteracting effect of antivenomous serum is possessed by other horse sera, such as the antidiphtheritic, antityphoid, antistreptococcic and antitetanic sera. That this is not so is shown by the following table:—

TABLE XIII.—*Cobra Poison*, 1 c.c. = ·2 mgrm.

·5 c.c.	C. P.	. . . . .	Complete H.
·5 ,,	,,	+ ·1 antistreptococcic serum . .	,,
·5 ,,	,,	+ ·5 ,, ,, . .	,,
·5 ,,	,,	+ ·5 antidiphtheritic ,, . .	,,
·5 ,,	,,	+ ·5 antityphoid ,, . .	,,
·5 ,,	,,	+ ·5 antitetanic ,, . .	,,
·5 ,,	,,	+ ·1 antivenomous ,, . .	No H.

Hence in the test-tube the action of a cobra antitoxin upon cobra toxin is as specific as it is in the animal body.

Correspondence between the Antihæmolytic Action in vitro *and the Protective Action of the Serum* in corpore.

For a guinea-pig, weighing 250–350 grms., ·1 mgrm. of fresh poison is the minimum certain lethal dose, death ensuing in from five to eight hours.[1] Now we have shown that the hæmolytic action of this quantity of poison was completely counteracted by ·1 c.c. of the isotonic antivenomous serum employed. And we found that when the ·1 mgrm. of poison was mixed with ·1 c.c. of isotonic antitoxin, so that the mixture had no hæmolytic power, it was never fatal to the animal. When incompletely neutralised the animal did or did not die. We may summarise our results in the following table:—

TABLE XIV.

		Animals taken (subcutaneous injection).	Deaths.
1	Mixtures of serum and poison which give no hæmolysis, *i.e.* ·1 mgrm. toxin + ·1 c.c. isotonic cobra antitoxin	12	0
2	Mixtures of serum and poisons which give hæmolysis, *i.e.* ·1 mgrm. toxin + less than ·1 c.c. isotonic serum	13	9
3	Poison controls ·1 mgrm. cobra toxin . . .	13	13

[1] Professor Fraser asserts that ·2 mgrm. per 1 kilo is the minimum lethal dose, but we have found that this is by no means a certain lethal dose.

The first group includes three cases where the amount of poison neutralised was ·15 mgrm. But when we took larger quantities we found that, although the poison was neutralised as regards hæmolysis, the animals died (five out of six).

Thus 3 c.c. cobra poison (= ·6 mgrm.) + ·6 c.c. isotonic serum was a non-hæmolysed mixture. Yet it was rapidly fatal to the animal. We see, then, that the correspondence only holds for the dose already mentioned; and does not obtain for multiples. We may explain this in the following way. The poison may contain, in addition to the toxic substance, which is neutralised by the antivenomous serum, a toxic substance which is not so neutralised. C. J. Martin ([13]) has recently drawn attention to the fact that many snake poisons, including cobra poison, contain at least two proteid substances, of which one is coagulable and indiffusible, the other is incoagulable and diffusible.[1]

C. J. Martin has further shown that whilst one of these substances is neutralised by the serum, the other is not. Now if this be the case, it is obvious that a single lethal dose mixed with the quantity of serum necessary to neutralise the hæmolytic poison would contain only a small quantity of the other poisonous substance, whilst six times this lethal dose would contain six times as much of this other poisonous substance, which would remain active. Thus if a single lethal dose of poison contain two substances (A and B), of which A is present in a single lethal dose, and B in sublethal quantity, then six lethal doses would contain 6 A + 6 B. Now 6 A are neutralised by the serum, while 6 B remain free and act fatally.

In a future communication we propose to deal with this side of the question; for the present we merely offer this explanation. Similar observations have been made by Wassermann ([16]) with regard to pyocyaneus toxin, and its antitoxic serum. He found that with four times the quantity of serum necessary to neutralise 1 c.c. of strong pyocyaneus toxin, he could not neutralise twice that quantity of toxin. This he explains by assuming that twice the poison is not neutralised *in vitro*, because the large quantity of toxin present paralyses the cells, so that the tissues cannot utilise the antitoxin and change it from the inactive to active form. However, he has made no attempts to analyse the pyocyaneus toxin, and presupposes that it contains a single toxic substance. It will be necessary in future to carefully analyse the toxins; for it is possible that many of these may contain several substances, some of which are not neutralised by the antitoxin. For ricin, abrin, diphtheria toxin, and

[1] Weir Mitchell and Reichert some years ago found that in cobra poison the proportion of coagulable to total proteid was 1·75 per cent. C. J. Martin ([15]) believes that so far as Pseudechis venom is concerned, the coagulable proteid is a hæmolytic poison, whilst the incoagulable one is principally a nerve cell poison. We are not prepared as yet to discuss this matter, but must reserve it for another time.

tetanus toxin, the law of multiples holds good; and this may be because these substances are purer than cobra poison and pyocyaneus toxin. We purpose to come back to this point on a future occasion.

Our experiments agree so closely with those of Ehrlich on ricin and antiricin that we are forced to come to the same conclusions as he did. They show that the antitoxic serum chemically acts directly upon the toxin, and that the neutralisation point in the test-tube agrees with that in the animal body (within the limits specified above). Cellular action being excluded in our test-tube reactions, it must be supposed that the antitoxins act chemically upon the toxin, even without the assistance of the living tissues.

We may briefly summarise our results as follows:—

1. Cobra poison is strongly hæmolytic *in vitro*.

2. This action is neutralised by antivenomous serum, and the action of the latter is specific.

3. For certain doses (·1 mgrm.) the measure of this neutralisation *in vitro* is a measure of the neutralisation *in corpore* for guinea-pigs.

4. The neutralisation is chemical, and not cellular or vital.[1]

Part II. The Influence of Cobra Poison on the Clotting of Blood and the Action of Antivenomous Serum upon this Phenomenon *in Vitro*.[2]

The experiments shown by Professor Kanthack to the Physiological Society at St. Bartholomew's Hospital in 1896, as stated in Part I. of this paper, established the fact that the delay in clotting occasioned by cobra poison, which had been described by D. D. Cunningham ([6]), could be counteracted by admixture of antivenomous serum, and did not occur with the blood of an immunised animal.

The following is an account of his experiments:—

(*a*) An ordinary normal rabbit was bled from the carotid artery under an anæsthetic.

3 c.c. of blood collected in a test-tube clotted in one minute.

3 c.c. of blood collected in a test-tube containing 4 mgrms. of cobra poison did not clot in three days.

(*b*) An immunised rabbit was then bled from the carotid artery under an anæsthetic.

3 c.c. of blood collected in a test-tube clotted in one to two minutes.

[1] Since the above was written, a communication by H. Kossel ([17]) appeared, in which he shows that the hæmolytic action of eel's serum can be neutralised *in vitro* by the addition of serum of a rabbit immunised against this eel poison, and this neutralisation strictly obeys the law of multiples *in vitro*. It further appears that recently Gley and Camus ([18]) have independently demonstrated the same phenomenon. Their complete paper as yet has not been published.

[2] Communicated before the Physiological Society, 7th May 1898.

3 c.c. of blood collected in a test-tube containing 4 mgrms. of cobra poison clotted in one to two minutes.

(*c*) An ordinary normal rabbit was bled from the carotid artery under an anæsthetic.

3 c.c. of blood collected in a test-tube clotted in one minute.
3 c.c. of blood collected in a test-tube containing 4 mgrms. of cobra poison was imperfectly clotted after twenty-four hours.
3 c.c. of blood collected in a test-tube containing 4 mgrms. of cobra poison and 1 c.c. of serum from an immunised rabbit, clotted fairly firmly after fifteen minutes, firmly after twenty-four hours.

In 1897 Professor Kanthack further proceeded with the inquiry whether the blood of an animal artificially immunised showed greater resistance to the power of cobra poison than that of a normal animal. Thus a kitten received 100 mgrms. of cobra poison, per os, in five doses of 20 mgrms. extending over nine days. Fifteen days after the last quantity of poison had been given the animal was bled.

A definite quantity of blood was collected in test-glasses containing poison of given strength. Examples of his results are the following:—

TABLE XV.

(*a*)	Saline + blood 10 c.c.	Clotting about equal in all three tubes—(*a*) clotted rather later than (*b*) and (*c*).
(*b*)	C. P. 2 mgrms. + blood 10 c.c.	
(*c*)	C. P. 4 mgrms. + blood 10 c.c.	

Two days later the antitoxic action of the cat's serum was tested on guinea-pigs by subcutaneous injection.

TABLE XVI.

C. P. ·1 mgrm.	Guinea-pig 340 grms.	Died 24 - 36 hours.
C. P. ·2 ,, + 1 c.c. serum . .	,,	No symptoms.
C. P. ·1 ,, + 1 c.c. ,, . .	,,	No symptoms.

The correspondence between these experiments *in vitro* and *in corpore* is manifest. Table XV. shows that the blood was able to resist as much as 4 mgrms. of cobra poison, a quantity that would inhibit partially or completely the clotting of normal blood; while Table XVI. shows that simultaneously the serum of the animal had acquired antitoxic properties.

At Professor Kanthack's suggestion we made use of this power of cobra poison to inhibit clotting as a test-tube reaction, and endeavoured to find out whether, as in the case of "hæmolysis," so also here the

antitoxin would neutralise this inhibitory action of the toxin *in vitro*, *i.e.* without the assistance of living cells.

Before proceeding to our own experiments we may quote a series of observations illustrating more accurately this inhibitory influence upon clotting by cobra poison, and the neutralising action *in vitro* of this antitoxin.

TABLE XVII.

1 vol. ,,	Blood. Saline solution.	7 minutes; firm clot.
,, ,, ,,	Blood. C. P. (1 c.c. = 1·0 mgrm.) Saline solution.	20 minutes; loose clot.
,, ,, ,,	Blood. C. P. (1 c.c. = 1·0 mgrm.) Antivenomous serum.	12 minutes; firm clot.
,, ,, ,,	Blood. C. P. (1 c.c. = 5·0 mgrms.) Antivenomous serum.	60 minutes; loose clot. 20 hours; solid clot.
,, ,, ,,	Blood. C. P. (1 c.c. = 20·0 mgrms.) Antivenomous serum.	60 minutes; no clot. 20 hours; solid clot.
,, ,,	Blood. C. P. (1 c.c. = 1·0 mgrm.)	Fairly solid clot in 18 hours.
,, ,, ,,	Blood. C. P. (1 c.c. = 1·0 mgrm.) Antitoxin.	Firm clot in 3 minutes.
,, ,, 3 vol.	Blood. C. P. (5 c.c. = 1·0 mgrm.) Antitoxin.	Firm clot in 10 minutes.
1 vol. ,, ,,	Blood. Saline solution. Antitoxin.	Firm clot in 4 minutes.
,, ,, ,,	Blood. C. P. (1 c.c. = 1 mgrm.) Saline solution.	No clot in 60 minutes.
,, ,, 3 vol.	Blood. C. P. (1 c.c. = 5 mgrms.) Antitoxin.	Firm clot in 4 minutes.
1 vol. ,, ,,	Blood. C. P. (1 c.c. = 5·0 mgrms.) Saline.	No clot in 24 hours.
,, ,, ,,	Blood. C. P. (1 c.c. = 5·0 mgrms.) Antitoxin.	Solid clot in 24 hours.

These observations show distinctly that cobra poison inhibits clotting *in vitro*, and that this inhibitory influence is removed by the addition of the specific antitoxin. A large number of observations have been made, all of which demonstrate the same fact, but the examples quoted must suffice.

(*b*) Our experiments were made with a view, as we have stated previously, of ascertaining how far the specific inhibitory action of the poison could be neutralised by its antitoxin *in vitro*. The difficulties in the way of satisfactorily determining the clotting time of a blood are great, and no really satisfactory method has been so far described; and we must confess that the method used in the experiments just recited, which consisted in mixing volumes of blood, saline solution, cobra poison, and antitoxin in a test-tube, is rather crude, and not suited for careful quantitive estimations. If we consider alone differences due to excess or deficiency of CO_2 or O in blood, of alkalinity, of excess or deficiency of leucocytes,—and in this relation digestion has a marked influence in quickening the clotting time,—of exposure of the blood to contact with foreign bodies, and difference in the extent to which the thoroughness of mixture of solutions is effected, we shall not be surprised that the values obtained at different times and in different ways do not agree. Still in any particular experiment these factors are sufficiently constant; so that the different values obtained may justly be attributed to some known disturbing cause, namely, in these experiments the solution of cobra poison. One of the simplest methods of determining the clotting time is to conduct the blood direct from the artery into the test solution. Thus, as an illustration of the inhibitory influence upon clotting of cobra poison solutions we have the following results:—

TABLE XVIII.—*Guinea-pig; Anæsthetised; Cannula inserted in Carotid,* 4.20 P.M.

		Clotting Time.	Next Day.
1.	1 c.c. C. P. (=20 mgrms.)+1 c.c. blood.	Black solution.	Fluid.
2.	,, =10 ,, + ,,	Black solution.	Fluid.
3.	,, = 5·0 ,, + ,,	1 hour; black clot.	Solid jelly; no serum.
4.	,, = 2·5 ,, + ,,	18 min.; black.	Solid; no serum.
5.	,, = 1·25 ,, + ,,	9 min.; black.	Solid; no serum.
6.	1 c.c. saline, 0·5 per cent. + ,,	6 min.; scarlet.	Contracted clot; 1 c.c. serum.
6A.	1 c.c. C. P. (= ·625 mgrm.)+ ,,	2 min.	...
7.[1]	,, = ·312 ,, + ,,	Deep red serum.	Semifluid clot.
8.[1]	,, = ·07 ,, + ,,	Bright red clot; red serum.	Semifluid clot.
9.[1]	,, = ·02 ,, + ,,	Bright red clot; clear serum.	Serum stained.
10.[2]	,, = ·004 ,, + ,,	Bright red clot; clear serum.	Serum stained.
11.[2]	,, = ·002 ,, + ,,	Bright red clot; clear serum.	Serum unstained.

[1] Indicates that a fresh guinea-pig was used for 7, 8, 9.
[2] Indicates that a fresh guinea-pig was used for 10, 11.

From this series it appears, then, that the influence of the poison on the immediate colour of the clot, to which Cunningham has drawn attention, had disappeared when solutions weaker than 1 c.c. = 1·25 mgrm. were used.

The hæmolytic action described in Part I., showing itself in the staining of the serum in these experiments, was observable with a strength of 1 c.c. = ·004 mgrm. For a large series of experiments made to show this action of the poison, reference must be made to Cunningham's paper already quoted.

In determining the interaction of poison and serum, other methods were, however, employed, in which it was unnecessary to use such relatively large volumes of poison and blood. A blood solution was prepared by diluting shed guinea-pig's blood with one-tenth its volume of a 10 per cent. sodium citrate solution in 10 per cent. salt. A known volume of the blood was then mixed with a known volume of poison solution, and to such a mixture traces of a 5 per cent. calcium chloride solution were added. The following (Table XIX.) is an example of this method :—

TABLE XIX.

·25 c.c. blood . ·5 c.c. saline, $CaCl_2$.	15 minutes ; clot. 24 hours ; solid.
·25 c.c. blood . ·5 c.c. C. P. (1 c.c. = ·2 mgrm.) $CaCl_2$.	No clot ; 3 hours. 24 hours ; gelatinous clot.
·25 c.c. blood . ·5 c.c. C. P. (1 c.c. = ·02 mgrm.) $CaCl_2$.	3 hours ; clot. 24 hours ; rather firm clot.
·25 c.c. blood . ·5 c.c. C. P. (1 c.c. = ·01 mgrm.) $CaCl_2$.	3 hours ; clot. 24 hours ; rather firm clot.
·25 c.c. blood . ·5 c.c. C. P. (1 c.c. = ·005 mgrm.) $CaCl_2$.	3 hours ; clot. 24 hours ; rather firm clot.

By this method the delay in clotting effected by a strength of poison 1 c.c. = ·005 mgrm. is well marked, and the difference in the resulting clot is also easily appreciable. Proceeding now to test the influence of serum on this action, we got the following results :—

TABLE XX.[1]

1.	·25 c.c. blood + 1·25 c.c. saline + $CaCl_2$.	10 min. ; nearly solid.
2.	,, ,, + (·5 c.c. C. P.) + ·75 c.c. saline + $CaCl_2$.	24 hours ; no clot.
3.	,, ,, + (·5 c.c. C. P. + ·05 isotonic serum) + ·75 c.c. saline + $CaCl_2$.	30 min. ; small clot.
4.	·25 c.c. blood + (·5 c.c. C. P. + ·075 isotonic serum) + ·75 c.c. saline + $CaCl_2$.	10 min. ; nearly solid.
5.	·25 c.c. blood + (·5 c.c. C. P. + ·1 c.c. isotonic serum) + ·6 c.c. saline + $CaCl_2$.	10 min. ; nearly solid.
6.	·25 c.c. blood + (·5 c.c. C. P. + ·2 c.c. isotonic serum) + ·55 c.c. saline + $CaCl_2$.	10 min. ; gelatinous clot.
7.	·25 c.c. blood + (·5 c.c. C. P. + ·3 c.c. isotonic serum) + ·45 c.c. saline + $CaCl_2$.	20 min. ; gelatinous clot.
8.	·25 c.c. blood + (·5 c.c C. P. + ·5 c.c. isotonic serum) + ·25 c c. saline + $CaCl_2$.	20 min. ; gelatinous clot.
9.	·25 c.c. blood + (·5 c.c. C. P. + ·75 c.c. isotonic serum) + $CaCl_2$.	30 min. ; gelatinous clot.
10.	,, ,, + (·2 c.c. undiluted serum) + 1·05 saline + $CaCl_2$.	45 min. ; nearly solid.

[1] The strength of the poison solution used was 1 c.c. = ·2 mgrm., and the serum was isotonic for guinea-pig's blood.

The strength of the poison solution used in this series was 1 c.c. = ·2 mgrms. It will be seen that in Experiment 2, using this solution, no clot was obtained even after twenty-four hours; while, for example, by the addition of ·1 c.c. of isotonic serum, clotting was normal, namely, in 10 minutes. Further, it will be noticed that the serum itself delays clotting somewhat. This fact we were also able to demonstrate by mixing blood and antivenom in test-tubes. Thus—

TABLE XXI.

1 vol.	Blood.	Firm clot in 1 minute.
2 vol.	Saline solution.	...
1 vol.	Blood.	...
,,	Saline solution.	Loose clot in 2–3 minutes.
,,	Antivenom.	Never firm.
,,	Blood.	Loose clot in 13–16 minutes.
2 vol.	Antivenom.	...

It is evident from this table that antivenom alone delays clotting, and to us this seems an important fact, for the assumed combination of two antipodal substances, both of which possess the power of delaying coagulation, although to an unequal degree, has completely lost this inhibitory influence upon clotting.

Finally, recourse may be had to a direct method, which, though giving variable results for two specimens of blood taken from the finger at the same moment, yet by making the conditions such that these variations were of no great import, it was possible to show readily that the poison could be counteracted by the serum. We simply used pieces of capillary tubing of equal calibre, graduated so that known volumes of poison, serum, and blood could be sucked up. The serum and poison mixtures were previously sucked up to a known mark, and then an equal volume of blood sucked from the finger, the contents being rapidly mixed by inverting the tubes several times. The clotting time was tested by observing—first, whether on inverting the tube the column of blood still moved about; secondly, when the clot could no longer be blown out of the tube. The observations were always made at ordinary temperature, as the object was only a comparative one (Table XXII.).

Using a poison 1 c.c. = ·625 mgrms., specimens of blood were kept fluid for over an hour almost invariably; while with a mixture consisting of ·5 c.c. C. P. = 1·25 + ·7 isotonic serum (a mixture which gave only a trace of hæmolysis) the clotting time was reduced to about the same as that of blood diluted with saline. A strong poison solution was chosen, as the difference could thus be clearly demonstrated. No attempt was made by this method to show a gradation

in result according to the proportionate amounts of serum and poison, as was shown in Part I., to hold good for hæmolysis. When poison solutions of half the strength were used, the clotting times were not sufficiently separated to exclude errors inherent in the method itself.

TABLE XXII.

I.	II.	III.	IV.
·5 c.c. C. P. = 1·25 + ·5 c.c. Saline.	·5 c.c. C. P. = 1·25 + ·7 c.c. Antivenomous Serum.	Antivenomous Serum (Isotonic).	Saline, 5 per cent.
60 minutes; still fluid . .	(1) 12 minutes; firm. (2) 17 ,, solid.	... ...	(1) 10 minutes. (2) 5 ,,
60 minutes; still fluid and very small clot .	(1) 3 ,, (2) 6 ,,	10 minutes.	9 ,,
60 minutes; still fluid . .	6 ,,	6 ,,	6½ ,,
24 minutes; still fluid . .	8 ,,	16 ,,	5 ,,
60 minutes; still fluid and slight clot . .	5½ ,,	7 ,,	10 ,, 7½ ,, Another specimen of blood.
60 minutes; still fluid and small clot	12 ,,	10½ ,,	8½ mins. (1) 10 ,, Second specimen of blood. (2) 18 ,,
60 minutes; still fluid, very small clot	12 ,,	11½ seconds.	11½ ,, 25 ,, Second specimen of blood.
60 minutes; still fluid, some clot	12 ,,	7½ minutes.	12 minutes.

Note.—When two figures are given in the table, the first signifies when the blood could be inverted, the second when it was solid.

The next question which arises is whether this neutralising action of the antitoxin is specific, or whether other sera are capable of producing the same effect.

It can be easily demonstrated that just as in the body the antitoxins react only upon the toxins or their toxoids (8), so also *in vitro* the cobra antitoxin reacts only upon cobra toxin.

The specific action of the serum could readily be shown in this way. Thus, working with the following mixtures—

(*a*) ·5 c.c. C. P. = 1·25 mgrms,
·8 ,, isotonic cobra antitoxin,

(*b*) ·5 ,, C. P. = 1·25 mgrms,
·8 ,, of diphtheria antitoxin,

on mixing these with blood there was at once an evident difference in the fluidity and laky appearance of *b* solution as contrasted with *a*, which showed no hæmolysis, and soon clotted. Thus not only with regard to hæmolysis, but also with regard to clotting, could we prove the specific action *in vitro* of cobra antitoxin for the same solutions (*a* and *b*). This is shown in Table XXIII.

TABLE XXIII.

	I.	II.	III.	IV.	V.	VI.
(*a*)	11½ min.; clot.	10 minutes; clot.	(1) 11½ minutes. (2) 16½ minutes; clot.	12 minutes; clot.	10 minutes; clot.	17 minutes; clot.
(*b*)	60 minutes; still fluid.	60 minutes; still fluid.	60 minutes; still fluid.	60 minutes; still fluid.	20 minutes, about; clot.	60 minutes; still fluid.

Diphtheria antitoxin, therefore, except in one instance, had no effect in counteracting the inhibitory action upon clotting of the poison.

Similarly, we made experiments which showed that the anti-venomous serum had no action in controlling the inhibitory action upon clotting of peptone solutions or of leech extract.

The experiments detailed in Table XXIII. were performed with capillary tubes according to the method above described. The specific action of cobra antitoxin upon the inhibitory influence upon clotting of cobra toxin can, however, be equally well shown by mixing known volumes of blood, poison, and serum in test-tubes. A number of observations are given in Table XXIV.

TABLE XXIV.

1 vol.	Blood.	
,,	C. P. (1 c.c. = 20 mgrms.)	No clot.
,,	Saline solution.	
,,	Blood.	
,,	C. P. (1 c.c. = 20 mgrms.)	Solid clot in 3 minutes.
,,	Antivenin.	
,,	Blood.	
,,	C. P. (1 c.c. = 20 mgrms.)	No clot.
,,	Normal rabbit serum.	
,,	Blood.	
,,	C. P. (1 c.c. = 20 mgrms.)	,,
,,	Diphtheria antitoxin.	
,,	Blood.	
,,	C. P. (1 c.c. = 20 mgrms.)	,,
,,	Antityphoid serum.	
,,	Blood.	
,,	C. P. (1 c.c. = 20 mgrms.)	,,
,,	Antistreptococcus serum.	
,,	Blood.	
,,	C. P. (1 c.c. = 20 mgrms.)	Slight clot in 30 minutes.
,,	Serum from partially immunised rabbit.	

The specific action of antivenomous serum on the inhibitory action upon clotting of cobra poison is clearly established by these series of experiments. We have thus demonstrated once more that the antitoxin possesses the power of acting upon the toxin *in vitro*, as well as *in corpore*, and that this action is as specific in the test-tube as it is in the body.

Now when studying the neutralising action of cobra antitoxin upon cobra toxin with regard to hæmolysis, we found that the neutralising point *in vitro* corresponded to the neutral point *in corpore*. It remains to be seen whether this is also true when clotting is used as the test reaction.

It is more difficult to apply the clotting method in a quantitative manner, since differences in the clotting time are not so easily recognised as degrees of hæmolysis. Nevertheless, it is possible by using test-tubes instead of capillary tubes, and larger quantities of blood and poison and antivenin to demonstrate that a mixture of blood and poison and antivenin which is exactly neutral, *i.e.* clots as quickly as the control specimen of blood alone, is also neutral for the animal. Several series of observations have been made on this point, but it will suffice for our purpose to quote one of these.

TABLE XXV.

1.	·25 c.c. blood + 1·25 c.c. saline + trace of $CaCl_2$.	Clotted in 10 mins.
2.	·25 c.c. ,, + ·5 c.c. C. P. (1 c.c. = ·2 mgrms.) + ·75 c.c. saline. + trace of $CaCl_2$.	No clot in 24 hours.
3.	·25 c.c. ,, + ·5 c.c. C. P. (1 c.c. = ·2 mgrms.) + ·05 c.c. antitoxin + ·75 c.c. saline. + trace of $CaCl_2$.	Small loose clot in half an hour.
4.	·25 c.c. ,, + ·5 c.c. C. P. same strength + ·075 c.c. antitoxin + ·75 c.c. saline. + trace of $CaCl_2$.	Nearly solid in 10 minutes.
5.	·25 c.c. ,, + ·5 c.c. C. P. same strength + ·1 c.c. antitoxin + ·65 c.c. saline. + trace of $CaCl_2$.	Nearly solid in 10 minutes.
6.	·25 c.c. ,, + ·5 c.c. C. P. same strength + ·2 c.c. antitoxin + ·55 c.c. saline. + trace of $CaCl_2$.	Gelatinous clot in 10 minutes.
7.	·25 c.c. ,, + ·5 c.c. C. P. same strength + ·3 c.c. antitoxin + ·45 c.c. saline. + trace of $CaCl_2$.	Gelatinous clot in 20 minutes.

It is evident from this table that, so far as clotting is concerned, ·1 mgrm. of cobra poison is neutralised by ·075—·1 c.c. of antitoxin *in vitro*; and excess of antitoxin renders the clot gelatinous. The antitoxin used was of the same strength as that used for the experiments in Part I.; and on referring to Table XIV. (Part I.), it will be seen that ·1 c.c. of antitoxin exactly neutralises ·1 mgrm. of cobra poison. We see, therefore, that there exists an exact correspondence between the neutral points *in vitro* (clotting being the test reaction) and *in corpore*.

Conclusions.

We may briefly summarise the results of the experiments described in this section as follows :—

1. Cobra poison delays or inhibits clotting of blood *in vitro.*

2. This inhibitory action upon clotting of cobra poison is neutralised by antivenomous serum *in vitro.*

3. This action of the antivenomous serum *in vitro* is specific.

4. The antivenomous serum itself, when added to blood, delays clotting.

5. For certain doses (·1 mgrm.) the measure of the neutralisation *in vitro,* using clotting as the test reaction, is also the measure of the neutralisation *in corpore* for guinea-pigs.

6. The neutralisation of the toxin by its antitoxin *in vitro* is certainly not vital or cellular, but must be chemical.

Our experiments, therefore, lend further support to Ehrlich's view that the antitoxin renders the toxin harmless by combining with it, for it is reasonable to assume that if in the test-tube the antitoxin binds the toxin, it is able to do the same in the body.

BIBLIOGRAPHY.

1. Buchner, ... *München. med. Wchnschr.*, 1893, Nos. 24 and 25, S. 449–452 and 480–483
2. Behring, ... "Bekämpfung der Infections-Krankheiten," Leipzig, 1894, S. 243.
3. Kanthack, ... "System of Medicine," edited by T. Clifford Allbutt, London, 1896, vol. i. p. 570.
4. Calmette, ... *Ann. de l'Inst. Pasteur*, Paris, 1895, tome ix. p. 251.
5. Wassermann, ... *Ztschr. f. Hyg.*, Leipzig, 1896, Bd. xxii. S. 312.
6. Cunningham, D. D., . . "Scientific Memoirs by Medical Officers of the Army of India," part 9, p. 1.
7. Ehrlich, ... *Fortschr. d. Med.*, Berlin, 1897, Bd. xv. No. 2.
8. „ . . . . . "Die Werthbemessung des Diphtherieheil-serums," *Abdruck aus dem Klin. Jahrb.*, Berlin, 1897, Bd. vi. S. 13.
9. Wassermann, ... *Berl. klin. Wchnschr.*, 1898, No 1, S. 4 and 5.
10. Hamburger, ... For references, see von Limbeck, "Grundriss der Klin. Pathologie des Blutes," Jena, 1896, Aufl. 2, S. 158–166 and S. 26 and 57.
11. Vaquez, ... *Compt. rend. Soc. de biol.*, Paris, 1897.
12. Fraser, ... "An Address at the Royal Institution," March 20, 1896, *Nature*, 1896, vol. liii. p. 571.
13. C. J. Martin, ... *Journ. and Proc. Roy. Soc. N. South Wales*, Sydney, Aug. 5, 1896 ; *Intercolonial Med. Journ. Australasia*, Aug. 20, 1897.

14. Weir Mitchell and Reichert — *Smithson. Contrib. Knowl.*, Washington, 1886, vol. xxvi.
15. C. J. Martin, ... — *Journ. and Proc. Roy. Soc. N. South Wales*, Sydney, Aug. 5, 1896.
16. Wassermann, ... — *Ztschr. f. Hyg.*, Leipzig, 1896, Bd. xxii. S. 312 and 313.
17. H. Kossel, ... — *Berl. klin. Wchnschr.*, 1898, Bd. xxxv. No. 7, S. 152.
18. Gley and Camus, . . . — *Semaine méd.*, Paris, Feb. 2, 1898.