

completely involved the gland and has left no trace of gland tissue. The next specimen (Fig. 18) I throw on the screen is on the table, No. 2836A. It was removed from the tail of the pancreas but the child succumbed shortly after

FIG. 17



Spindle-celled sarcoma of the pancreas.

the operation. Operation for sarcoma of the pancreas is uncommon, though the few cases operated on prove that if the tumour be in the tail of the pancreas the case is one amenable to surgical treatment. I explored the abdomen in one case of the kind but found the disease too extensive

FIG. 18.



Sarcoma removed from the tail of the pancreas.

for removal. Kronlein in 1894 removed a tumour of the size of the fist but the patient died seven days later. A tumour which was successfully removed by Briggs proved to be sarcomatous degeneration of an echinococcus cyst.

Mr. President, I must apologise for the imperfect manner in which I have been able to deal with the several subjects in my last lecture but want of time has compelled me to cut out much of what I should like to have said. I hope, however, that my lectures will be the means of exciting more general interest in the surgery of the pancreas, a subject which I feel is much more worthy of attention than hitherto has been accorded to it. It only remains for me to thank you for the patient hearing and the kindly interest that have been shown in what I have had to say and at the same time I would thank the Council of the Royal College of Surgeons of England for the honour they have done me in electing me a third time to hold this important chair.

ON THE PRECIPITIN OF COBRA VENOM.

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SECOND COMMUNICATION.

IN a previous communication¹ on the above subject it was shown that the serum of a rabbit which had been subjected to gradually increasing doses of pure unheated cobra venom possesses the property of causing a coagulum or precipitum when mixed in suitable proportions with solutions of suitable strength of this venom; that, in short, the rabbit reacts to injections of the proteids of cobra venom exactly in the same way—that is, by the formation of specific precipitins—in which it reacts to injections of various other proteid substances, such as the proteids of serum, egg albumen, Witte's peptone, &c. It was further demonstrated that when this immune serum and a solution of cobra venom of definite strength were mixed in suitable proportions both the precipitin and the precipitable proteids were entirely used up. Taking advantage of this fact we were able to give a numerical expression to the precipitin value of the serum in terms of the amount of cobra venom which a given quantity of serum would exactly neutralise. It was on this account suggested that by this method we might possibly have at hand an easy and ready means of standardising an antivenomous serum. In conclusion, it was shown in this communication that while the serum of a rabbit immunised with cobra venom gave with the proteids of daboia venom a copious precipitum, indistinguishable, in fact, from the precipitum given with cobra venom itself, no coagulum was obtained when this serum was mixed with the venoms of three other species of snakes—namely, (1) *echis carinata*, (2) *bungarus fasciatus*, and (3) *hoplocephalus curtus*.

In the present communication it is proposed further to extend these observations in several directions which were indicated in the previous paper. It is, however, as well to mention at the outset that several of the problems which were then mentioned as awaiting solution have still to be left undecided. In the first place, the observations summarised above have been extended by testing a cobra venom serum—that is to say, the serum of a rabbit highly immunised with pure unheated cobra poison—with the venoms of five other species of snakes—namely, (1) *naja bungarus*; (king cobra); (2) *bungarus cœruleus* (common krait); (3) *enhydrina valakadien* (a common sea snake); (4) *trimeresurus gramineus* (green pit viper); and (5) *crotalus adamanteus* (Californian rattlesnake).² In order to make these observations as conclusive as possible two methods of technique were employed in each instance. The first method consisted in mixing different proportions of serum with one part of venom solution of definite strength—namely, 0.05 per cent. In the second method three parts of serum were mixed with one part of venom solution of different strengths. In all cases these mixtures were effected by means of Wright's tubes of large calibre. The preparations were then kept at from 35° to 37° C. for about 20 hours, when the results were recorded. A reference to the protocols (Tables I. and II.) will show that while no coagulum was formed with three of the poisons mentioned above—namely, the venoms of *naja bungarus*, of *bungarus cœruleus*, and of *crotalus adamanteus*—and only a trace of precipitum with the venom of *enhydrina valakadien*, a marked precipitum was obtained with the poison of *trimeresurus gramineus*. This precipitum, however, was not nearly so copious as those got with cobra venom itself and with daboia venom. Taking the above results along with those which were contained in my previous communication we can divide the venoms which have been tested with a serum prepared by injecting rabbits with pure unheated cobra poison into three groups as follows:—

GROUP A. *Venoms which give a copious precipitum.*—(1) Venom of *naja tripudians* (cobra); and (2) venom of *vipera Russellii* (daboia).

¹ THE LANCET, August 16th, 1902, p. 431.

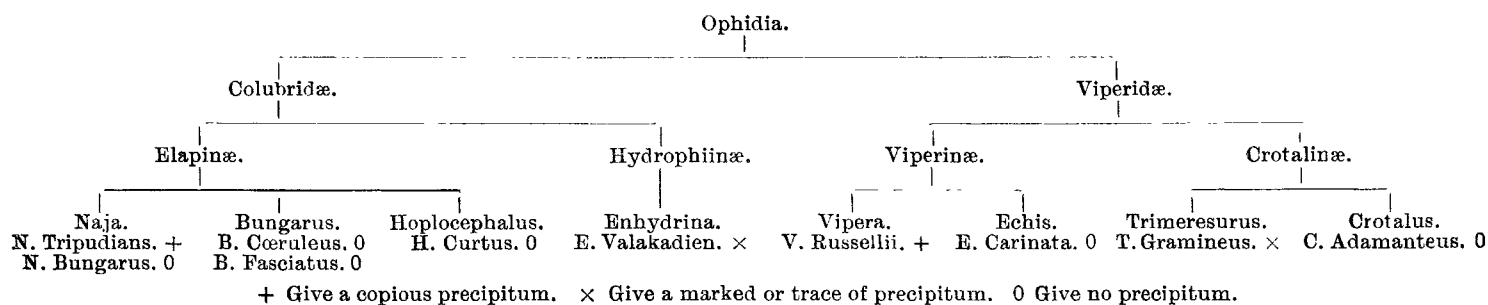
² The king cobra venom was obtained from two snakes the property of the Natural History Society, Bombay; the sea-snake venom from Mr. Peal, Calcutta; and the crotalus venom from Professor S. Flexner, Philadelphia. I am much indebted for these gifts. The other poisons were collected under my own supervision.

GROUP B. *Venoms which give a marked or trace of precipitum*.—(3) Venom of *trimeresurus gramineus* (green pit viper); and (4) venom of *enhydrina valakadien* (common sea snake).

GROUP C. *Venoms which give no precipitum*.—(5) Venom of *naja bungarus* (king cobra), (6) venom of *bungarus coeruleus* (common krait), (7) venom of *bungarus fasciatus* (banded krait), (8) venom of *hoplocephalus curtus* (tiger snake), (9) venom of *echis carinata* (phoorsa), and (10) venom of *crotalus adamanteus* (Californian rattlesnake).

With a view of seeing if the venoms which give a precipitin reaction with a cobra venom immune serum are derived from species which are more closely related in the animal kingdom to the cobra than are the species the poisons of which do not give this reaction, the accompanying diagram, which shows at a glance the position of these several species among the ophidia, has been constructed.

DIAGRAM SHOWING THE PRECIPITIN REACTION IN DIFFERENT SPECIES.



A study of this diagram will at once make it apparent that this venom precipitin test affords no indication of the closeness of species relationship. Thus, for example, it will be noted on the one hand that the venom of *naja bungarus*, a species belonging to the same genus as the cobra, gives no precipitum with a cobra venom immune serum, and, on the other hand, that the venom of *vipera Russellii*, a species belonging even to a different family from the cobra, gives a copious precipitum with cobra venom immune serum. These observations therefore are not in accord with the similar observations which have been made by Nuttall and others working with immune sera prepared by injections of the sera of different species of animals and which went to show that a serum prepared by injections of the serum of one species of animal gave a certain amount of precipitum with the sera of animals closely related to the animal the serum of which was used for the injections and gave no precipitum with the sera of animals more distantly related. The same law does not evidently hold good for the venoms of the poisonous snakes. Further, these results show that great differences must exist between the proteids of the poisons of the several species of snakes, differences which must be kept carefully before the mind when dealing with the physiological actions of the venoms of the several species.

In the second place, I have now to pass on to the consideration of some observations which were made with the serum of the cobra itself and with the serum of a rabbit which had been treated by repeated injections of cobra serum. The cobra serum was obtained by opening the thorax of living snakes, cutting the aorta across, and catching the blood from the cut end. After allowing the blood to clot a clear serum was got by centrifugalisation. The first series of experiments was made with a view of ascertaining if cobra serum contained any precipitin for cobra venom. Two methods of experimentation were employed. In one method four parts of serum were mixed with one part of cobra venom solution of a strength varying from 1 per cent. to 0.0078 per cent. In the other method varying proportions of the serum were brought in contact with one part of cobra venom solution of fixed strength—namely, 0.05 per cent. The proportions of serum to venom solution varied from 10 to 1 to 1 to 2. As no trace of coagulum was obtained in any of these preparations the experiments are not given in detail in the protocols.

Such a result was only to be expected. It has been shown by several observers that the cobra is immune to its own poison and that this immunity is not due to the presence of any antitoxic substances in its blood. It is therefore evident that, although constant absorption of the poison must be taking place, neither antitoxin nor precipitin is prepared. Stating the conclusion to be drawn from these facts in the

language of Ehrlich's side-chain theory, we can say that the cobra's cells must be deficient in the specific receptors which are present in the cells of animals susceptible to the poison and which allow of the poison being bound to the cells. It would be interesting in this connexion to ascertain whether the central nervous tissues of the cobra were able to neutralise the poison in vitro in the same manner as has been shown in the case of those tissues of susceptible animals and also whether the cobra's red cells were susceptible to the poison in the same way as the red cells of other animals—that is to say, were hæmolyzed by the venom. Unfortunately, however, I have no living cobras at present at my disposal.

The second series of observations now under consideration was made with cobra venom immune serum and cobra serum. In these experiments also two methods of technique were employed. In one method the cobra venom immune

serum was mixed in varying proportions with one part of cobra serum. These proportions varied from 10 to 1 to 1 to 1. In the other method four parts of cobra venom serum were mixed with one part of different dilutions of cobra serum, the dilutions varying from two-fold to 1024-fold. In both instances the cobra venom immune serum was the serum of a rabbit highly immunised with cobra venom. Not a trace of coagulum was observed in any of these preparations. We can therefore conclude that the serum of a rabbit highly immunised with cobra venom contains no precipitin for cobra serum.

The third series of experiments was made with a view of ascertaining if the serum of a rabbit which had been treated with injections of cobra serum contained any precipitin for this serum as well as for daboia serum. A rabbit was treated with three subcutaneous injections of fresh cobra serum. These injections, amounting to 11 cubic centimetres in all, were made at intervals of about ten days. The serum of the rabbit was collected eight days after the last injection. It was then tested against both cobra serum and daboia serum, the latter of which was obtained in the same manner as has been described in the case of the cobra serum. A reference to the protocols (Tables III. and IV.) will show that a very copious and well marked precipitum was obtained with cobra serum, while a much less marked but still distinct reaction was obtained with daboia serum. The difference between these two reactions was very apparent and is well shown by the fact that in many of the preparations when a very copious coagulum was observed with cobra serum only opacity without any distinct sedimentation was seen in the corresponding tubes made with daboia serum.

It was now of interest to ascertain if this rabbit's serum, prepared with cobra serum, gave any precipitin reaction with cobra venom. An experiment made with this end in view is detailed in Table V. of the protocols. It will be seen from this table that a well-marked precipitin reaction took place between these two substances. The reaction, however, was not so well marked as that between cobra venom immune serum and cobra venom. It is difficult in the present state of our knowledge as regards proteid precipitin and proteid assimilation to reconcile the two facts now demonstrated—namely, that a serum prepared by injections of cobra venom contains no precipitin for cobra serum but that a serum prepared by injections of cobra serum gives a well-marked precipitin with cobra venom. I am afraid I can at present offer no suitable explanation of the phenomena.

In my previous communication on the subject under discussion I was able to show that, working with the serum of a rabbit immunised with cobra venom, it was possible to give a numerical expression to the precipitin value of this serum by estimating with what proportions of serum and

venom solution of known strength both the precipitin and precipitable substances were used up. Further, it was suggested that by such an estimation of the precipitin value of a serum a method of standardising an antivenomous serum might be elaborated. In the third place, I have now to put on record some observations which show definitely that the hope which was then expressed cannot be fulfilled. In the course of these experiments some interesting points were settled as regards the relationship of precipitin to antitoxin. These points will also now receive attention. The first experiments were made with the serum of an ass which had received over a period of six months repeated injections of pure unheated cobra venom. The antitoxic value of this serum was estimated by experiments on rats after the method already described.³ A reference to the protocols (Table VI.) will show that one cubic centimetre of this serum was able to neutralise 0.07 milligramme of cobra venom. On experimenting now to ascertain the precipitin value of the serum it was found that it contained no, or only the merest trace of, precipitin (*vide* protocols, Table VII.). This result at once suggested that the formation of antitoxin is independent of the formation of precipitin. As, however, the antitoxic value of this serum was by no means high, it was necessary to make further experiments with a serum of much greater antitoxic power. Further observations were therefore made with the serum of a horse (Horse A) which had been treated over a period of nearly two years with repeated subcutaneous injections of pure unheated cobra venom. The antitoxic value of this serum was determined in a manner similar to that used in the case of the serum of the ass, small rabbits, however, being employed instead of rats. It will be seen from the protocols (Table VIII.) that one cubic centimetre of this serum was able to neutralise at least 1.2 milligrammes of cobra venom, an antitoxic value nearly 20 times greater than that of the serum of the ass. An attempt was next made to estimate the precipitin value of the serum. If the results obtained in these experiments (protocols, Tables IX. and X.) be compared with the results of the estimation of the precipitin content of the serum of a rabbit highly immunised with cobra venom (protocols, Tables XIV.-XVII.) it will be at once seen that this horse's serum contained evidently such a small amount of precipitin that there was no possibility of making an accurate estimation of it after the manner I have already described.⁴ First, it appears from these two observations that neither the ass nor the horse, in comparison with the rabbit, is a suitable animal for the preparation of a precipitin for cobra venom. This conclusion is substantiated by experiments which were made with the serum of another horse (Horse B) which had been subjected over a period of 15 months to repeated injections of pure unheated cobra venom. The serum of this horse was also found to contain very little precipitin. As the experiments gave practically the same results as those obtained with the serum of Horse A they are not detailed in the protocols. It is only necessary to add that the reaction was not quite so well marked as in the case of the serum of Horse A, but it was sufficient to show that a certain, though small, amount of precipitin was present. Secondly, it appears from these two observations that the elaboration of precipitin is quite independent of the formation of antitoxin which results from injections of pure cobra venom. To this point we shall return in a moment. It is now, however, evident from these two facts—namely, that the horse, the animal most suitable for the preparation of an antitoxic serum, reacts only slightly to the injection of cobra venom by the formation of specific precipitin and that the formation of precipitin seems to be quite independent of the formation of antitoxin—that the suggestion I previously put forward that this precipitin test might furnish us with a method of standardisation of antivenomous sera cannot be realised.

A further proof of the fact that the formation of precipitin is quite independent of the elaboration of antitoxin is afforded from the examination of the serum of a horse (Horse C) which had been treated for a period of 22 months by repeated injections of pure cobra venom heated for half an hour at 72° C. The antitoxic value of this serum, as estimated on small rabbits (*vide* protocols, Table XI.), was found to be considerable, one cubic centimetre of serum being able to neutralise about one milligramme of unheated cobra venom.

On testing for the presence of precipitin in this serum it was found to contain practically none of this substance (protocols, Tables XII. and XIII.). Further, I tested the sera of two horses which were in the process of immunisation also with pure cobra venom heated for half an hour at 72° C. One of these horses had been under treatment for a period of six months and the other for a period of three months. Neither of these sera contained even a trace of precipitin. Now we have seen above that the serum of a horse immunised with unheated cobra poison contains precipitin, although the quantity, in comparison with the amount present in the serum of an immunised rabbit, is small. It would appear, therefore, from these last observations that when the solution of venom is heated at 72° C. before injection the elaboration of precipitin does not take place in the case of the horse. In conclusion, I have now to bring forward the result of an observation which also bears on the question of the elaboration of precipitin being independent of that of antitoxic substances inasmuch as it consists of some estimations made at intervals of the amount of precipitin present in the serum of a rabbit which was treated over a period of 19 months with gradually increasing doses of pure unheated cobra venom.

Two methods were used to form an estimate of the amount of precipitin in this serum. Linossier and Lemoine,⁵ as a result of a long series of experiments, pointed out that in order to give a numerical expression to the precipitin value of a serum it was only necessary to ascertain at what maximum dilution the precipitable proteids begin to be precipitated under the action of a known precipitin, the proportion of serum to the various dilutions remaining the same in all the preparations. On this principle one of the methods employed in my experiments was founded. Further, I also used the method described in my previous communication—namely, the method of estimating with what proportion of serum and venom solution of known strength a neutral solution was obtained; that is to say, a solution containing neither precipitin nor precipitable substances. The first precipitin estimation of this rabbit's serum was made after the animal had been under treatment for a period of five months, during which period it had received a total amount of 0.0151 gramme of venom. The last injection made before this observation was one of 0.0018 gramme—that is, about six lethal doses. It will be seen from the protocols (Table XIV.) that at this period of immunisation when three parts of serum were mixed with one part of venom solutions of different strengths a coagulum began to appear with a solution of a strength of 0.00625 per cent. Further, it will be seen (Table XV., *a* and *b*) that the neutralisation point was reached when six parts of serum were mixed with one part of venom solution of 0.05 per cent. strength. Various observations were made at intervals after this primary estimation, but at no time was any increase of this precipitin value of the serum found to be present. At the end of 19 months when the rabbit had received as a last dose 0.008 gramme, equivalent to about 26 lethal doses, and a total amount of 0.151 gramme of venom, the precipitin value of the serum remained practically the same as it was 14 months previously (protocols, Tables XVI. and XVII.). If anything it had slightly decreased. Thus, when three parts of serum were mixed with venom solutions of different strengths only a mere trace of coagulum was observed in the preparation containing a solution of 0.00625 per cent. strength and the neutralisation point was reached when eight parts of serum were mixed with one part of venom solution of 0.05 per cent. strength. From these observations we can conclude that the precipitin value of the serum of a rabbit undergoing immunisation with pure unheated cobra venom quickly reaches a maximum and remains at or about this level during the process. Although the antitoxic value of this particular serum was not at any time estimated, we can take it from *a priori* reasons and from the facts that the animal stood well the gradually increasing doses of venom and was at the time of the last experiment in a condition of high immunisation, that the serum was at the end of the process of high antitoxic value. These observations therefore lend support to the conclusion arrived at already—namely, that the elaboration of the precipitin of cobra venom is quite independent of the formation of true antitoxin.

PROTOCOLS.

In all the precipitin experiments detailed hereafter the mixtures were effected by means of Wright's sedimentation

³ Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India. New Series. 1902. No. 1.

⁴ THE LANCET, August 16th, 1902, p. 431.

⁵ Comptes Rendus de la Société de Biologie, tome liv., 1902, pp. 85, 276, 320, and 369.

tubes of large calibre. The tubes after being sealed were kept at from 35° to 37° C. for 20 hours, when the results were recorded. In all the tables the following signs are used: + signifies a copious precipitum; × signifies a marked precipitum; * signifies a slight or trace of precipitum; † signifies opacity without any precipitation; and 0 signifies no precipitum or opacity.

TABLES I. AND II.—*Experiments with Cobra Venom Serum and other Varieties of Snake Venom.*

The serum which was used was that of a rabbit which had been highly immunised by subcutaneous injections of pure unheated cobra poison. The snake venoms were obtained from (1) *naja bungarus* (king cobra) = N.B.V.; (2) *bungarus cœruleus* (common krait) = B.C.V.; (3) *enhydrina valakadien* (common sea snake) = E.V.V.; (4) *trimeresurus gramineus* (green pit viper) = T.G.V.; and (5) *crotalus adamanteus* (Californian rattlesnake) = C.A.V. Two series of experiments were made. In the first series varying proportions of serum were mixed with one part of a 0·05 per cent. solution of each poison. The following were the results obtained:—

TABLE I.

Proportion of serum to venom solution (0·05 per cent.)	N.B.V.	B.C.V.	E.V.V.	T.G.V.	C.A.V.
12-1	0	0	0	*	0
10-1	0	0	0	*	0
8-1	0	0	0	×	0
6-1	0	0	*	×	0
4-1	0	0	*	*	0
2-1	0	0	*	*	0
1-1	0	0	0	*	0

In the second series of experiments three parts of serum were mixed with one part of a solution of different strengths of each variety of poison as indicated below. The dilutions of venom were made with normal saline solution. The following were the results obtained:—

TABLE II.

Serum = 3 parts; venom solution = 1 part.

Strength of venom solution per cent.	N.B.V.	B.C.V.	E.V.V.	T.G.V.	C.A.V.
0·2	0	0	—	×	0
0·1	0	0	*	×	0
0·05	0	0	*	*	0
0·025	0	0	*	*	0
0·0125	0	0	*	*	0
0·00625	0	0	0	0	0
0·003125	0	0	0	0	0

TABLES III. AND IV.—*Experiments with the Serum of a Rabbit treated with Cobra Serum and Cobra and Daboia Sera.*

A rabbit was treated with three subcutaneous injections of two cubic centimetres, four cubic centimetres, and five cubic centimetres of cobra serum at intervals of ten days. Eight days after the last injection its serum was collected. Two methods of technique were employed. In the first series of observations varying amounts of the treated rabbit's serum were mixed with one part of both cobra serum and daboia serum. The following was the result:—

TABLE III.

Proportion of rabbit's serum to snake serum.	Cobra serum.	Daboia serum.
8-1	+	†
6-1	+	†
4-1	+	†
2-1	+	†
1-1	+	†

In the second series of observations three parts of the treated rabbit's serum were mixed with one part of increasing dilutions of both cobra serum and daboia serum. The dilutions were made with normal salt solution. The following results were obtained:—

TABLE IV.

Treated rabbit's serum = 3 parts. Diluted snake serum = 1 part.

Dilutions of cobra or daboia serum.	Cobra serum.	Daboia serum.
2 fold	+	†
4 "	+	†
8 "	+	×
16 "	+	×
32 "	+	*
64 "	×	*
128 "	×	*
256 "	×	*
512 "	*	0
1024 "	*	0

TABLE V.—*Experiment with the Serum of a Rabbit treated with Cobra Serum and Cobra Venom.*

The same rabbit's serum as was used in the previous observations was employed. Varying amounts of serum were mixed with one part of a cobra venom solution of fixed strength (0·05 per cent.). The following results were obtained:—

Parts of treated rabbit's serum.	Parts of cobra venom solution (0·05 per cent.).	Result.
8	1	*
6	1	*
4	1	+
2	1	×
1	1	×
1	2	×

TABLE VI.—*Experiments to Estimate the Antitoxin Value of the Serum of an Ass treated with repeated injections of pure unheated Cobra Venom.*

The ass had received over a period of six months repeated injections, gradually increasing in amount, of pure unheated cobra venom. The animals used for testing the serum were rats of about 115 grammes in weight. The minimum lethal dose of cobra venom for a rat of this size is about 0·05 milligramme. The test dose of venom used was 0·25 milligramme—that is to say, five lethal doses. This test dose and varying amounts of serum were mixed in vitro and allowed to stand for half an hour at laboratory temperature (25° C.). The mixtures were injected subcutaneously. The following was the result:—

Animal.	Cobra venom in milligrammes.	Serum.	Result.
Rat 1	0·25	10 c.c.	Died in four hours.
" 2	"	20 "	" 24 "
" 3	"	25 "	" 44 "
" 4	"	30 "	} No symptoms.
" 5	"	35 "	
" 6	"	40 "	
" 7	"	45 "	
" 8 (control) ...	"	Nil.	Died in three hours.

From the above series of experiments we can conclude that three cubic centimetres of serum could neutralise at least 0·25 — 0·04 = 0·21 milligramme of venom—that is, that one cubic centimetre could neutralise 0·07 milligramme.

TABLE VII.—Experiment to Show the Precipitin Reaction of the Serum of an Ass treated with repeated Injections of pure unheated Cobra Venom.

The serum used was the same as that employed in the previous series of observations. The method now employed to test the precipitin reaction was that method in which different proportions of serum are mixed with one part of 0.05 per cent. solution of cobra venom. The following was the result obtained :—

Parts of serum.	Parts of cobra venom (0.05 per cent.)	Result.
10	1	0
8	1	0
6	1	0
4	1	0
2	1	0
1	1	0
1	2	*

TABLE VIII.—Experiments to Estimate the Antitoxin Value of the Serum of Horse A.

This horse had been treated over a period of nearly two years with repeated subcutaneous injections, gradually increasing in amount, of pure unheated cobra venom. The test dose of cobra venom, left unheated, now used was two milligrammes for rabbits of about 650 grammes in weight—namely, about ten lethal doses. The minimum lethal dose, by subcutaneous injection, for rabbits of this sample of venom was about 0.35 milligramme per kilogramme. The test dose and different amounts of serum were mixed in vitro and the mixtures were allowed to stand for half an hour at laboratory temperature. They were then injected subcutaneously. The following were the results :—

Animal.	Weight (grammes).	Cobra venom in milli-grammes.	Serum.	Result.
Rabbit 1	610	2	1.0 c.c.	Died in 2½ hours.
„ 2	680	„	1.3 „	„ 12 „
„ 3	615	„	1.4 „	„ 13½ „
„ 4	720	„	1.5 „	No symptoms.
„ 5	685	„	1.6 „	„

From these experiments it will be seen that 1.5 cubic centimetres of serum neutralised 2 — 0.2 (maximum non-lethal dose for rabbit of 720 grammes) = 1.8 milligrammes. Therefore one cubic centimetre could neutralise 1.2 milligrammes.

TABLES IX. AND X.—Experiments to Show the Precipitin Reaction between the Serum of Horse A and Cobra Venom.

The same serum as was used for the experiments detailed above was employed to test the precipitin reaction. Two methods of technique were employed. In the first series of experiments varying proportions of serum were mixed with one part of a 0.05 per cent. solution of cobra venom. The following results were obtained :—

TABLE IX.

Parts of serum.	Parts of cobra venom solution (0.05 per cent.).	Result.
10	1	0
8	1	0
6	1	0
4	1	*
2	1	+
1	1	*
1	2	†

were mixed with one part of cobra venom solution of different strengths. The following was the result :—

TABLE X.

Parts of serum = 3 ; parts of C. V. solution = 1.

Strength of cobra venom solution.	Result.
0.2 per cent.	*
0.1 „	+
0.05 „	*
0.025 „	0
0.0125 „	0
0.00625 „	0
0.003125 „	0

TABLE XI.—Experiments to Estimate the Antitoxin Value of the Serum of Horse C.

This horse had been treated over a period of 22 months with gradually increasing doses of pure cobra venom heated for half an hour at 72° C. The technique used for this estimation was the same as was used in the experiments detailed in Table VIII. The following were the results :—

Animal.	Weight (grammes).	Cobra venom in milli-grammes.	Serum.	Result.
Rabbit 1	750	2	1.0 c.c.	Died in 3 hours.
„ 2	580	„	1.3 „	„ 3 „
„ 3	500	„	1.6 „	„ 18 „
„ 4	780	„	1.8 „	No symptoms.
„ 5	700	„	2.0 „	„

From these experiments it will be seen that 1.8 cubic centimetres of serum neutralised 2 — 0.2 = 1.8 milligrammes of venom. Therefore one cubic centimetre could neutralise one milligramme.

TABLES XII. AND XIII.—Experiments to Show the Precipitin Reaction between the Serum of Horse C and Cobra Venom.

The same serum as was used in the experiments detailed above was now employed to test the precipitin reaction. Two methods of technique were employed. In the first series of experiments varying proportions of serum were mixed with one part of a 0.05 per cent. solution of cobra venom. The following results were obtained :—

TABLE XII.

Parts of serum.	Parts of cobra venom solution (0.05 per cent.)	Result.
10	1	0
8	1	0
6	1	
4	1	
2	1	0
1	1	Slight opacity.

In the second series of experiments three parts of serum were mixed with one part of cobra venom solution of different strengths. The following was the result :—

TABLE XIII.

Parts of serum = 3 ; parts of C. V. solution = 1.

Strength of C. V. solution.	Result.
0.2 per cent.	
0.1 „	Slight opacity
0.05 „	0
0.02 „	0
0.0125 „	
0.00625 „	0

In the second series of experiments three parts of serum

From these observations it can be concluded that this serum contained practically no precipitin.

TABLES XIV. TO XVII.—Experiments to Estimate the Amount of Precipitin in the Serum of a Rabbit at Different Stages in the Process of Immunisation with Cobra Venom.

The rabbit was treated over a period of 19 months with subcutaneous injections of gradually increasing doses of pure unheated cobra venom. The first estimation of the precipitin content of the serum was made after the rabbit had been under treatment for five months, having received during this time 0.0151 gramme of venom. Two methods of appraising the precipitin value of the serum were employed. 1. In the first instance three parts of serum were mixed with one part of venom solution of different strengths. The following were the results :—

TABLE XIV.

Parts of serum = 3 ; parts of C.V. solution = 1.

Strength of venom solution.	Result.
0.1 per cent.	+
0.05 "	+
0.025 "	+
0.0125 "	+
0.00625 "	*
0.003125 "	0

2. In the second instance varying proportions of serum were mixed with one part of venom solution of fixed strength (0.05 per cent.). The following result was obtained :—

TABLE XV. (a).

Parts of serum.	Parts of venom solution (0.05 per cent.).	Result.
12	1	+
8	1	+
6	1	+
4	1	+
2	1	×
1	1	×

The clear supernatant fluid in each of these tubes was then obtained by simply filing off the end of the tube with the contained precipitum. It was divided into two portions. To one portion was added an equal quantity of cobra venom solution (0.05 per cent.) and to the other portion was added an equal amount of the same serum as was used in the first series of experiments. The results were as follows :—

TABLE XV. (b).

Proportion of serum to venom solution in original preparation.	C.V. solution (0.05 per cent.).	Serum.
12-1	*	0
8-1	*	0
6-1	0	0
4-1	0	*
2-1	0	×
1-1	0	+

It is evident, then, that the neutralisation point was reached when six parts of serum were mixed with one part of the venom solution (0.05 per cent.). The last estimation of the precipitin value of this rabbit's serum was made after it had been under treatment for 19 months, having received during this period 0.151 gramme of venom. The same method was used to estimate the precipitin value of the serum as in the previous observation. The results were as follows.

TABLE XVI.

Parts of serum = 3 ; parts of C.V. solution = 1.

Strength of venom solution.	Result.
0.1 per cent.	×
0.05 "	+
0.025 "	*
0.0125 "	*
0.00625 "	*
0.003125 "	0

TABLE XVII. (a).

Parts of serum.	Parts of cobra venom solution (0.05 per cent.)	Result.
12	1	+
10	1	+
8	1	+
6	1	+
4	1	×
2	1	×
1	1	*

The supernatant fluid in each tube was treated in the same manner as in the experiments detailed above (Table XV., a). The following was the result :—

TABLE XVII. (b).

Proportion of serum to venom solution in original preparation.	C.V. solution (0.05 per cent.)	Serum.
12-1	*	0
10-1	*	0
8-1	0	0
6-1	0	*
4-1	0	*
2-1	0	*
1-1	0	×

It is evident, then, that the neutralisation point was, in this case, reached when eight parts of serum were mixed with one part of venom solution (0.05 per cent.)

Kasauli, India.

ON A NEW METHOD OF TESTING THE BLOOD AND THE URINE,

WITH SPECIAL REFERENCE TO THE DETERMINATION OF THE EXCRETORY EFFICIENCY OF THE KIDNEY.

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THE examination of the urine for albumin is a very indirect, ineffective, and often fallacious method of obtaining information with regard to the excretory efficiency of the kidney. While it is capable of revealing leakage of the albuminous substances from the blood into the urine it tells us nothing with regard to the excretion of the salts and products of metabolism generally into the urine. This information is furnished by comparative cryoscopic examinations of the urine and of the blood from which that urine has been elaborated. These examinations, involving as they do not only resort to complicated and delicate apparatus but