T H E S I S

on

THE PRESENCE IN BLOOD SERA OF SUBSTANCES WHICH HAVE AN ACTIVATING OR INHIBITORY EFFECT ON THE HAEMOLYTIC PROPERTIES OF COBRA VENOM

- presented by -

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THE PRESENCE IN BLOOD SERA OF SUBSTANCES WHICH HAVE AN ACTIVATING OR INHIBITORY EFFECT ON THE HAEMOLYTIC PROPERTIES OF COBRA VENOM.

Medical Science on the experimental side has been productive of no more interesting or fruitful results than those which have appeared in connection with the study of Haemolysis. The groundwork of this subject was laid by Bordet (I) in 1900, by the discovery of the fact that an immune serum contains a thermostable substance (immune body) which, in the presence of the original immunising agent (blood corpuscles or bacteria), is capable of absorbing complement; that is to say, a haemolytic or bacteriolytic serum heated to 57°C. for an hour will lose its power of lysing the homologous blood or organisms, but it will retain thermostable substances whose activity can be restored by the addition of fresh non-immune serum. The absorption or destruction of complement by the immune body (antibody) in the presence of the original immunising agent (antigen) can be demonstrated by the subsequent addition of sensitised red blood/

blood corpuscles, that is, red blood corpuscles which have already been brought into contact with their specific inactivated immune serum, and only require the addition of complement to complete the haemolytic process. To take a concrete example; the fresh serum of an animal immunised against the cholera vibrio, if brought into contact with the organisms, produces lysis; the same serum, heated at 57°C. for an hour, fails to do so; if, however, fresh normal serum be added to the heated serum, bacteriolysis occurs; and, as Muir and Browning (II) have pointed out, if sufficient quantities of immune body and organisms be used, the haemolytic complement of the fresh serum will be absorbed; whether or not the complement has been absorbed can be easily determined by the subsequent addition of sensitised red blood corpuscles which require only the complement of a fresh serum to become lysed; thus, if after the addition of the sensitised red blood corpuscles, no haemolysis occurs, then a corresponding antigen (cholera vibrio) and antibody must have absorbed the complement; if, on the other hand, haemolysis has occurred, there cannot have been an association of antigen with its specific immune body.

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It was subsequently demonstrated by Gengou (III) that these phenomena presented themselves not only when organised elements such as blood corpuscles and bacteria were employed, but also in the case of the higher proteids; that is to say, the blood serum of an animal (antigen or precipitinogen) if brought into contact with its corresponding antiserum obtained from an animal of another species, will give a precipitate at the line of junction, and this precipitate can absorb haemolytic complement, as may be demonstrated by the absence of haemolysis of sensitised red blood corpuscles which have been left for 1½ hours in contact with the precipitate at 37°C. The later investigations of Moreschi (IV), Neisser and Sachs (V), and Muir and Martin (VI) have proved the extreme delicacy of this method of differentiating blood sera of various kinds, and the method now occupies an important and indispensable place in departments of forensic medicine and physiological chemistry. It has further been shown by Wassermann and Bruck (VII) that extracts or solutions of bacteria in organs (e.g. in the spleen in typhoid fever) in the presence of the homologous immune sera, are capable of absorbing haemolytic complement, and the recognition of this fact led Wassermann (VIII) to the application of the same principle in the diagnosis of syphilis, in which case he employed/

employed as antigen a solution or extract of the liver of a congenital syphilitic. Although it has been fully demonstrated by Marie and Levaditi (IX), Sachs and Altmann (X), Browning and MacKenzie (XI) and others, that the syphilitic reaction is not an antigen - antibody reaction in the strict sense of the term, the value of the test in the diagnosis of syphilis is beyond dispute, and it is now regarded by experienced observers as the most definite and reliable serum test employed in clinical medicine. Another branch of the subject of haemolysis is that which deals with the action of cobra venom on red blood corpuscles and it is this aspect of the subject with which the present investigation deals. Flexner and Noguchi (XII) in an elaborate examination of the haemolytic properties of cobra venom found that although red blood corpuscles whose serum had been completely removed by washing with salt solution were agglutinated by the venom, they were not dissolved; if, however, serum were added to the washed blood cells, or if unwashed blood were used, then haemolysis occurred. From this Flexner and Noguchi concluded that the haemolytic action of the snake venom is due to two factors; one of the components is contained in the snake venom itself, and is

said/

said to be capable of enduring an exposure to heat at about 90°C.; the other factor is a component of the serum, and this is believed to activate the poison which by itself is incapable of producing haemolysis; the conclusion to which these workers came was in effect, that cobra venom is made up of a number of substances, acting after the manner of immune bodies which are activated by certain complements of the serum. This important discovery stimulated further interest in the mode of action of cobra venom, and Kyes (XIII) working in Ehrlich's laboratory was able to demonstrate further the similarity which exists between haemolysis by immune serum and snake poison, respectively. He also showed that there are two kinds of blood cells so far as their behaviour in the presence of snake poison is concerned: —

(1) Those which are dissolved by cobra venom alone.

(2) Those that become dissolved only after the addition of other substances (complements, &c.).

These two groups were found to be comprised as follows:- the blood cells of the guinea-pig, dog, rabbit, man and horse are dissolved by cobra venom alone/

alone, while those of the ox, sheep and goat require the addition of serum or other activating substances. Again the blood cells of the first group do not all possess the same vulnerability, but manifest considerable variations depending on the species to which they belong, and in addition different specimens of blood from the same species vary in susceptibility to the action of the venom. With regard to such blood cells as require in addition to the venom, sera or other substances to complete the haemolytic process, Kyes points out that the sera which act as activators are of two kinds:-

(1) Certain sera lose their activating power when heated to 57°C. and thus behave like true complements; such phenomena present themselves in the following combinations:-

Horse blood. . . . . ox serum.

Ox blood. . . . . .guinea-pig serum.

Sheep blood. . . . . guinea-pig serum.

Rabbit blood. . . . . .guinea-pig serum.

(2) Other sera preserve their activating power when heated to 60°C., and in some cases the activating power is enhanced by heating to 65°C, while in other cases/

cases the activating power manifests itself only when the serum is heated to 65°C or 70°C. Such reactions are present in the following combinations:-

Ox blood. . . . . .human serum.

Ox blood. . . . . .ox serum.

Pursuing his investigations further, Kyes showed that the thermostable activating substances in sera and in animal tissues generally pass over into alcohol and ether, and that in a mixture of serum and alcohol the activating substances are in solution while the precipitate contains inhibitory substances. It was thus concluded that the thermostable activators are of the nature of phosphatides, and it was found that pure lecithin acted as an activator in very attenuated dilutions.

Investigation of the red blood cells which lyse in the presence of cobra venom alone (guinea-pig, man, &c.) led Kyes to the conclusion that in such cases haemolysis occurs through the presence of activators in the cells themselves, and these activators he designates as endocomplements. Further chemical examination tended to show that in such cases a "desponible lecithin" might be present, which, in the presence of cobra venom, completes the haemolytic process.

Reference/

Reference has already been made to the fact that in forensic medicine, clinical medicine and physiological chemistry, the reactions in a haemolytic system have been applied with far reaching results. Much and Holzmann (XIV) have recently published an article in which they suggest the possibility of a successful application of the cobra venom haemolytic reactions in the department of psychological medicine. That a definite chemical reaction might be present in such cases is to be expected, in as much as numerous observers, and among them Kraepelin, have contended for years that mental disease is not a functional disturbance pure and simple, but the manifestation of abnormal metabolic changes which may have their seat in various parts of the body; and the fact that such a reaction is said to be present in a type or types of mental disease, viz:- dementia precox and circular insanity, where up till now no evidence of organic change has been discovered, makes an examination of the conclusions of these observers all the more interesting and imperative. The reaction described by Much and Holzmann is based on the fact that cobra venom is capable by itself of lysing human red blood corpuscles/

corpuscles; the addition of serum from patients suffering from dementia precox or circular insanity, or epilepsy associated with circular insanity, prevents the occurrence of lysis, while sera from other sources have no such effect. In carrying out the experiments to be described, the technique detailed by Much was followed with slight modifications. In Much's experiments 0,35 c.c. serum was mixed with 0,25 c.c. solution of cobra venom (1 : 5000) and to this was added 0,5 c.c. washed human red blood corpuscles 10%; this was allowed to stand 2 hours at 37°C and was then placed overnight on ice, the results being then taken; inhibition of haemolysis constitutes a positive reaction; complete haemolysis or marked haemolysis is taken as negative. He summarises his results as follows:-
In the mental diseases designated by Kraepelin dementia precox and maniac depressive insanity, substances are present in the blood, which cannot be demonstrated in the blood from other nervous cases or in normal blood. These substances are present in very small amount and are demonstrable only by means of a biological reaction. It is not yet clear whether they belong to the cholesterin group. It is not possible by means of the reaction to distinguish between dementia precox and maniac depressive insanity; on the other hand these

two/

two conditions are by means of the reaction, sharply marked off from such apparently allied conditions as neurasthenia, hysteria, imbecility, idiocy, senile dementia and general paralysis. The reaction is present in the blood of individuals who at the time show no evidence of maniac depressive insanity, but who belong to families with a predisposition to mental disease. The reaction is absent where cerebro-spinal fluid is used instead of blood serum. When the cases are selected strictly according to the clinical classification of Kraepelin, 100 per cent. of those suffering from dementia precox and maniac depressive insanity give a positive reaction.

In the experiments carried out with a view to examining these conclusions, the following procedure was adopted: human red blood corpuscles were washed free of serum by centrifugalising three times with normal saline solution (0.85% Na Cl), and a standard 5% suspension of the corpuscles was obtained by making 3.2 c.c. of the washed sediment up to 100 c.c. with normal saline. The cobra venom solution was made in the proportion of 1 mg. to 1 c.c. normal saline (1 : 1000). On an average it was found at from 0,02 c.c. to/

to 0,04 c.c. of this solution sufficed to lyse 1 c.c. of a 5% suspension of human corpuscles. Tubes were put up with graduated amounts of human serum, and to each tube 0,04 c.c. of cobra venom solution was added, and to this was added 1 c.c. of a 5% suspension of corpuscles; the tubes were placed at 37°C. for two hours and were then kept in an ice chest overnight, the results being read next morning. With each series, the dose of cobra venom was estimated for the corpuscles used. (Table I).

T A B L E I.

Cobra venom 1/1000	Human serum.	Human Corpuscles 5%.	Haemolysis.
0·04 c.c.	0.2 c.c.	1 c.c.	trace
0·04 c.c.	0.3 c.c.	1 c.c.	0
0.04 c.c.	0.4 c.c.	1 c.c.	trace

Human corpuscles 5%. .	1 c.c.	1 c.c.	1 c.c.	1 c.c.	1 c.c.
Cobra venom 1/1000. .	0.01	0.02	0·03	0.04	0·05
Result[x].	0	t	c	c	c

This example shows a positive reaction, the human serum having inhibited the haemolytic action of 0.04 c.c. of cobra venom (1/1000), where the actual haemolytic dose of the venom was 0,03 c.c.

It/

x In the various tables the extent of the haemolysis is represented thus: 0 = no lysis; ft = faint trace; t = trace; m = marked; ac = almost complete; c = complete.

It is obvious that the haemolytic dose of venom for the test corpuscles is an all-important factor in the experiment. In accordance with the observation of Much it was found that a considerable variation existed in the sensitiveness of different corpuscles; and it was thus necessary to employ corpuscles in which lysis took place with an amount of venom just under the amount used in the experiments.

If the test corpuscles were particularly sensitive then in the experiments all the tubes might show lysis even when some of the sera possessed inhibitory properties. The following table gives an indication of the difficulty encountered in such a condition. Eight sera were examined with the blood of four of the specimens from which the sera were obtained.

T A B L E II.

	Cobra venom 1/1000				
Test Blood Corpuscles.	.01	.02	.03	.04	.05
24.	ac	c	c	c	c
25.	0	0	0	0	c
26.	0	0	0	c	c
27.	0	0	ac	c	c

Here it is seen that the vulnerability of the different/

different corpuscles presents considerable variation. The corpuscles from case 24 are almost completely lysed by 0·01c.c. of the venom solution, while those from case 25 require five times that amount to produce lysis. In cases 26, and 27, lysis is complete with 0·03 c.c. of the venom solution. These four sets of corpuscles were next employed in testing the inhibitory properties of eight different sera, with the following results:-

TABLE III.

Test corpuscles from Case 24 + venom (1/1000) 0·04 c.c. + sera.

	0·2 c.c.	0·3 c.c.	0·4
Serum 24. . . .	c	ac	ac
" 25. . . .	c	c	c
" 26. . . .	c	c	c
" 27. . . .	c	c	c
" 28. . . .	a	ac	ac
" 29. . . .	c	c	c
" 30. . . .	c	c	c
" 31. . . .	c	c	c

Test/

TABLE III (Contd.)

Test corpuscles from Case 25 + venom (1/1000) 0·04 c.c. + sera.

	0·2 c.c.	0·3 c.c.	0·4 c.c.
Serum 24. . .	t	0	0
" 25. . .	c	c	c
" 26. . .	t	0	t
" 27. . .	c	c	m
" 28. . .	0	0	0
" 29. . .	t	0	0
" 30. . .	c	ac	ac
" 31. . .	c	ac	ac

Test corpuscles from Case 26 + venom (1/1000) 0·04 c.c. + sera.

	0·2 c.c.	0·3 c.c.	0·4 c.c.
Serum 24. . .	t	t	t
" 25. . .	.c	c	c
" 26. . .	.t	t	t
" 27. . .	.c	c	c
" 28. . .	.t	0	t
" 29. . .	.t	t	t
" 30. . .	.c	c	c
" 31. . .	.c	c	c

Test/

TABLE III. (Contd.)

Test corpuscles from Case 27 + venom (1/1000) 0·04 c.c. + sera.

	0·2 c.c.	0·3 c.c.	0·4 c.c.
Serum 24.	t	0	t
" 25.	c	c	c
" 26.	m	t	t
" 27.	c	c	c
" 28.	t	0	0
" 29.	t	0	t
" 30.	c	c	c
" 31.	m	c	c

The experiments show that the corpuscles from Case 24 which are most vulnerable to the action of the venom, fail, when the usual amount of venom solution (0·04 of 1/1000) is employed, to showing the inhibiting action of the various sera. On the other hand the corpuscles whose lytic dose is 0·04 or 0·05 show a distinct difference in the various sera; sera 24, 26, 28 and 29 possess distinct inhibitory properties, while sera 25, 27, 30 and 31 do not.

On the other hand blood corpuscles were found which showed no trace of lysis with 0·05 c.c. of the venom solution, but these same corpuscles were completely lysed with 0.04/

0·04 c.c. of venom solution in the presence of 0·2 c.c., 0·3 c.c. and 0·4 c.c. of various sera, some of which exhibited marked inhibitory action when tested with corpuscles which lysed in the presence of 0·03 c.c. venom solution alone. Table IV gives the result of an experiment showing this apparently anomalous phenomenon.

T A B L E IV.

5% solution of corpuscles (Case 46) + cobra venom (1/1000) 0·04 + sera.

(With venom solution alone 0·05 showed no lysis).

	0·2 c.c.	0·3 c.c.	0·4 c.c.
Serum 41.	c	c	c
" 42.	c	c	c
" 43.	c	c	c
" 44.	c	c	c
" 45.	c	c	c
" 46.	c	c	c
" 47.	c	c	c
" 48.	c	c	c
" 49.	c	c	c
" 50.	c	c	c

5%/

TABLE IV. (Contd.)

5% solution of corpuscles (Case 47) + cobra venom (1/1000) 0·04 + sera.

(With venom solution alone 0·03 showed complete lysis).

	0·2 c.c.	0·3 c.c.	0·4 c.c.
Serum 41.	c	c	c
" 42.	t	0	0
" 43.	m	c	c
" 44.	c	c	c
" 45.	t	t	t
" 46.	t	0	0
" 47.	c	ac	ac
" 48.	t	t	0
" 49.	t	m	m
" 50.	c	c	c

The preceding table shows that the corpuscles from Case 46 were quite unsuitable for elucidating any difference in the sera as regards their inhibitory properties, that in fact the corpuscles behave not like human corpuscles but like ox corpuscles, in that they became lysed only on the addition of sera. On the other hand the corpuscles from Case 47 showed a variation in the properties of these same sera. The lytic dose/

dose of the venom was 0·03 c.c., and to each tube 0·04 c.c. was added. When the various sera were added it was seen that Nos. 42, 45, 46 and 48 possessed distinct inhibitory properties while the others showed no inhibition. It is thus clear that in the matter of technique care must be taken to accept conclusions only in cases where such corpuscles are employed as show differences in the properties of the sera in the presence of the standard amount of cobra venom solution. When the precautions referred to have been taken, an examination of human sera from various sources shows the presence of inhibitory properties in a certain proportion. The sera from 100 cases were examined, and of these 80 were from cases of mental disease, while of the remaining 20, three were normal. Table V gives the result of the experiments:-

T A B L E V.

A. Nervous cases.

Dementia precox	20	cases	-	12	positive	6	negative	2 doubtful.
Maniac depressive Insanity	10	"	-	4	"	4	"	2 "
General Paralysis.	20	"	-	9	"	7	"	4 "
Epilepsy.	15	"	-	6	"	7	"	2 "
Idiocy and Imbecility.	15	"	-	6	"	8	"	1 "

B/

TABLE V. (Contd.)

B. Cases without nervous symptoms.

Pneumonia.	4	cases	- 1	positive	2	negative	1	doubtful.
Scarlet Fever.	6	"	- 2	"	3	"	1	"
Enteric Fever.	4	"	- 1	"	3	"	0	"
Phthisis.	3	"	- 2	"	1	"	0	"
Normal.	3	"	- 1	"	1	"	1	"

When one reviews generally the results of the investigation it is obvious that, for diagnostic purposes, the test does not answer the claim which has been made for it by Much. The cases were classified according to the system adopted by Kraepelin and now generally accepted, and there can be little doubt but that a positive reaction does not appear in all cases of dementia precox and maniac depressive insanity; and it is also equally true that the reaction is present in cases which do not present symptoms of mental disease. Much asserts, however, that its presence may be proved also in cases which at the time show no signs of mental disease, but which have a hereditary predisposition in that direction. The evidence obtainable in the cases examined, does not admit of a confirmation or refutation of this conclusion, but apart from that, the occurrence of a positive reaction in so many cases, not suffering from nervous disease, renders the reaction quite useless as a means of confirming/

firming a clinical diagnosis. It might on the other hand be argued from the above results, that the reaction is more frequent in nervous cases, but the number of sera examined is too small to admit of such a generalisation.

Although the reaction did not seem to have any clinical significance, the phenomena were interesting and merited further investigation. A series of experiments were performed with a view to determining whether the presence or absence of inhibitory properties bore any relation to the capacity which the various sera possessed, in activating the haemolytic power of cobra venom for ox corpuscles. Six sera were chosen, four of which possessed distinct inhibitory properties and two of which did not. Ox red blood corpuscles were sensitised by added cobra venom in the proportion of 1 c.c. of a 1 : 1000 solution of venom to 10 c.c. of a 5% suspension of ox corpuscles, and Table VI shows the result of the experiment.

TABLE VI.

Ox corpuscles 5% + cobra venom ($\frac{1}{10}$ mg : 1 c.c. corpuscles) + sera.

		Sera.				
		0·5 c.c.	0·3 c.c.	0·2 c.c.	0·1 c.c.	0.05 c.c.
Serum 51	Much positive	0	0	0	0	0
" 53	" "	0	0	0	0	0
" 54	" "	0	0	0	0	0
" 60	" "	0	0	0	0	0
" 52	Much negative	c	c	c	c	ac
" 56	" "	c	c	c	av	m

It is thus seen that those sera which possess a strong activating power for the action of cobra venom on ox corpuscles do not give evidence of having an inhibitory effect on the action of cobra venom on human corpuscles; while those sera which have an inhibitory influence on the lytic action of venom on human corpuscles have no activating power for the venom action on ox corpuscles even in considerable doses.

It was pointed out by Kyes that lecithin has an activating effect on cobra venom for ox corpuscles, and it was further shown by Kyes and Sachs (XV) that cholesterin has an inhibitory influence on the activating property of lecithin. The results obtained by experiments with lecithin and cholesterin have led some observers to the conclusion that/

that blood sera possess activating and inhibitory properties in virtue of their content in lecithin and cholesterin respectively. In view of this contention experiments were carried out to determine whether the inhibitory properties of the human sera prevented the activation of cobra venom by lecithin with ox corpuscles. Table VII shows the result of such an experiment.

T A B L E VII.

Lecithin (1·7% solution). 1 : 5	Serum (51) 0·3 c.c.	Ox corpuscles + C.V. added immediately.	Ox corpuscles + C.V. added after 1½ hours at 37°C.
0·005		0	0
0·01		trace	trace
0.02		almost complete.	almost complete.
0·03		complete	complete
0·05		"	"
0·075		"	"
0·1.		"	"

The activating dose of the lecithin for the sensitised ox corpuscles in the absence of serum was 0·03 c.c. of the solution used; and the serum (No. 51) in an amount of 0·3 c.c. was capable of inhibiting the action of a lytic dose of cobra venom solution on human corpuscles.

It/

It is thus probable at least, that the inhibiting property of the serum is not due to the presence of cholesterin. On the other hand it was considered possible that an inhibiting serum might inactivate an activating serum although incapable of inhibiting the activating power of a lecithin solution, and this actually proved to be the case as shown in Table VIII. For the carrying out of this experiment sera 51 and 52 were used. As seen from Table VI, serum 51 with a dose of 0·5 c.c. possessed no activating properties for cobra venom with ox corpuscles while serum 52 produced complete lysis in the amount of 0·1 c.c. Serum 51 inhibited the lytic action of venom on human corpuscles while serum 52 did not.

T A B L E VIII.

Serum 52	Serum 51	Ox corpuscles + C.V.
0·2	0·1	Almost complete.
0·2	0·2	trace.
0·2	0·3	0
0·2	0·4	0

Thus it is seen that although the inhibitory properties of serum 51 did not manifest themselves in the case of a lecithin activation, (Table VII), they did inhibit the activating power of serum 52. The conclusion/

conclusion is thus suggested, that the activating properties of a serum may not be due to its lecithin content, and the activating lecithin obtained from the serum by alcoholic extraction may be quite different from the substances in virtue of which the serum itself produces activation.

With a view to further investigation of this proposition, the properties of ox serum and guinea-pig serum were examined. Ox serum in doses up to 0·5 c.c. fails to produce venom activation for ox corpuscles, while it is capable of inhibiting the lytic action of venom on human corpuscles; in this respect it resembles such human sera as give a positive Much reaction. Guinea-pig serum on the other hand activates cobra venom in amounts down to 0·02 c.c. and in this respect resembles certain human sera which give a negative Much reaction. Certain characteristics of guinea-pig serum in which it differs from human serum will be referred to later.

It could be shown (Table IX) that ox serum is capable of inhibiting the activating properties of guinea-pig serum for cobra venom.

TABLE IX.

Guinea-pig serum.	Ox serum 0·2 c.c.	Ox corpuscles + C.V.
0·02.		No lysis.
0.04		"
0·06.		"
0.08		"
0·1.		Faint trace.

Thus while 0.02 c.c. fresh guinea-pig serum is capable of producing lysis of 1 c.c. of a 5% suspension of ox corpuscles to which 0.1 c.c. of a 1 : 1000 solution of cobra venom has been added, 0·2 c.c. of ox serum is able to inhibit the activating power of 0·1 c.c. of fresh guinea-pig serum for the same sensitised suspension. It has already been pointed out by Kyes and Sachs that cholesterin does not inhibit the activating power of fresh guinea-pig serum, although it inhibits the action of lecithin; this is in accord with the suggestion indicated above that the inhibiting substance in serum is probably not cholesterin, and if the inhibiting substance be not cholesterin there is no experimental grounds for the belief that the activating substance is lecithin. It has, however, been contended by Noguchi, Calmette, Kyes and Sachs that in the case of fresh guinea-pig serum, the activation occurs as a result of the/

the presence of haemolytic complement; in other words this is regarded as a case of true complementing, just as lysis is produced by fresh guinea-pig serum when brought into contact with ox corpuscles which have been sensitised with the homologous immune body of the rabbit. This contention has been supported by the fact, that guinea-pig serum loses its activating power when heated for an hour at 57°C., and also that cholesterin does not inhibit the activating power of fresh guinea-pig serum for cobra venom. These facts suggested a further examination of guinea-pig serum with reference to the variations which it exhibits in its activating power, when heated to different temperatures. In the first place the inhibitory influence of ox serum on the activation by fresh guinea-pig serum was tested, both in the case of corpuscles sensitised with cobra venom and also with corpuscles sensitised with homologous immune body. The result of such an experiment is shown in Table X.

TABLE X.

Fresh Guinea-pig serum.	5% ox corpuscles + 1.B. + 0·2 c.c. ox serum.	5% ox corpuscles + C.V. + 0.2 c.c. ox serum.
0·02 c.c.	Lysis complete.	No lysis.
0.04 c.c.	" "	" "
0.08 c.c.	" "	" "
0·1 c.c.	" "	" "

Dose of fresh guinea-pig serum for ox corpuscles + homologous immune body (1.B.) = 0·01 c.c.

Dose of fresh guinea-pig serum for ox corpuscles + cobra venom (C.V.) = 0·02 c.c.

This experiment demonstrates the fact that while fresh guinea-pig serum appears in some respects to act like a true complement in the activation of cobra venom, still in the presence of ox serum the lytic power on ox corpuscles sensitised with immune body is preserved, while its lytic power on ox corpuscles sensitised with cobra venom is lost.

It has been observed that, while guinea-pig serum loses its activating power for cobra venom when heated for an hour at 57°C., it regains the power of activation when heated again for an hour at 65°C. Experiments were next performed with a view to testing whether guinea-pig serum inactivated by heating, possessed inhibitory/

hibitory properties. It was found that, when the serum was heated for an hour at 57°C., it no longer activated cobra venom, but it did not exercise an inhibitory influence on the activating power of fresh guinea-pig serum. On the other hand, when heated for twelve hours at 57°C., or for one hour at 60°C., guinea-pig serum exercises a marked inhibitory effect on the activating power of fresh guinea-pig serum. Such an experiment is shown in Table XI.

T A B L E XI.

Fresh guinea-pig serum.	5% ox corpuscles + 1.B. + 0·1 c.c. guinea-pig serum 1 hour at 60°C.	5% ox corpuscles + C.V. + 0·1 c.c. guinea-pig serum 1 hour at 60°C.
0·02 c.c.	Lysis complete.	No lysis.
0·04 c.c.	" "	" "
0·08 c.c.	" "	" "
0·1 c.c.		" "

Dose of fresh guinea-pig serum for ox corpuscles + homologous immune body (1.B.) = 0.01 c.c.

Dose of fresh guinea-pig serum for ox corpuscles + cobra venom (C.V.) = 0·02 c.c.

This experiment confirms what was seen in Table X, that the activating power of fresh guinea-pig serum for cobra venom can be inhibited without destroying the/

the complementing effect of the serum for corpuscles sensitised with homologous immune body. It would thus appear as if the contention put forward by Noguchi, Calmette, Kyes and Sachs, that fresh guinea-pig serum acts as a true complement in cobra venom activation, broke down under this experiment. In any case it is clear, that the addition of ox serum or of guinea-pig serum heated to 60°C renders one property inert without interfering with the other.

It has been noted that ox serum markedly inhibits the lytic action of cobra venom, and also neutralises sera which have an activating effect on the venom. Fresh guinea-pig serum possesses strong activating properties; heated to 57°C. it appears to be neutral, while heated for an hour at 60°C. it possesses distinct inhibitory properties; heated further at 65°C. it regains its activating power. These properties of ox and guinea-pig serum resemble those which were noted in human sera.

In the case of human sera it was demonstrated that the activating properties of one serum could be neutralised by the inhibiting properties of another, while a serum with inhibitory properties did not affect the activating power of lecithin. In the case of the sera

of/

of the ox and guinea-pig those with inhibitory properties neutralise those with activating properties, and while cholesterin does not affect the activating power of fresh guinea-pig serum, ox serum does possess an inhibitory effect. It would thus seem as if the activating and inhibitory properties of sera were not associated with their content in lecithin and cholesterin respectively.

IV. Ox serum possesses inhibitory properties to those possessed by inhibitory human serum pig serum, while possessing strong activat when fresh, is strongly inhibitory when hea hour at 60°C.

V. The inhibitory properties of ox serum

C O N C L U S I O N S.

I. Human sera vary considerably in the possession of properties which exercise an inhibitory or activating influence on cobra venom haemolysis.

II. The fact that some sera inhibit and others activate cobra venom, does not possess, so far, any clinical significance.

III. A serum with inhibitory properties can neutralise the effect of a serum with activating properties, but does not neutralise the activating power of lecithin.

IV. Ox serum possesses inhibitory properties similar to those possessed by inhibitory human sera; guinea-pig serum, while possessing strong activating powers when fresh, is strongly inhibitory when heated for an hour at 60°C.

V. The inhibitory properties of ox serum and of guinea-pig serum heated for an hour at 60°C. can neutralise the activating effect of fresh guinea-pig serum.

VI. This neutralisation of the activating power of fresh/

fresh guinea-pig serum for cobra venom does not influence its complementing action on corpuscles sensitised with homologous immune body.

VII. Although Cholesterin does not neutralise the activating power of fresh guinea pig serum, this is accomplished by the addition of ox serum or of guinea-pig serum heated for an hour at 60°C. It would thus appear as if Cholesterin were not the body which exercised the inhibitory influence.

VIII. There are thus good grounds for believing that sera do not activate or inhibit in virtue of a lecithin or cholesterin content; and it may be doubted whether true complementing occurs with fresh guinea-pig serum and cobra venom in the sense in which it takes place when fresh serum in brought into contact with an antigen and its homologous immune body.

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