(NEW SERIES.)

No. 17.

SCIENTIFIC MEMOIRS

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OF THE

GOVERNMENT OF INDIA.

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BY

CAPTAIN GEORGE LAMB, M.D., I.M.S.

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SNAKE-VENOMS IN RELATION TO HÆMOLYSIS.

IN recent years the study of the action of snake venoms in relation to hæmolysis has acquired considerable importance in view of the results of the researches of Flexner and Noguchi[1] in America and of Kyes and Sachs[2] working in Ehrlich's laboratory in Germany. While by these researches a flood of light has been thrown on the intimate mechanism of venom hæmolysis, it has also been shown that this phenomenon is analogous in many respects to serum hæmolysis and is therefore worthy of the most careful investigation in consideration of the relationship which exists between this latter process and bacteriolysis. As an introduction to this communication it will be convenient, therefore, to give a brief summary of the results of the investigations of the workers mentioned above, in so far as these results concern our present purpose.

Flexner and Noguchi were the first to point out that washed blood corpuscles are not hæmolysed by venom, but that, if the separated serum is restored to each of the several kinds of blood corpuscles treated with venom, lysis takes place. They also showed that the serum of another species may act as the activating element in this process, but that such a foreign serum does not act so well as the homologous serum. They, therefore, concluded that venoms in the process of hæmolysis act only as intermediary bodies or amboceptors, requiring a complement to complete the reaction. Further, working with the washed corpuscles of the dog, of the rabbit and of the guinea pig, they state that a venom solution treated in succession with these corpuscles gives up to each a part of its intermediary body. In these experiments they do not particularise the venoms which were used nor the temperature at which this union was effected. It is to this question that I wish to refer especially in this paper.

In a later communication these authors elaborate their previous researches in several directions. They show that fresh snake venom contains no complement: they also enter into several intricate problems with reference to the relationship between snake venom amboceptor and complement and with reference to a comparison of the haptophore groups of snake venom and snake serum intermediary bodies. These problems do not concern us at the present moment. In a third paper, however, entitled, "On the plurality of cytolysins in snake-venom," they, among other details, state that a venom, to wit that of the water mocassin, when kept for 3 hours at 0° C. in contact with the red corpuscles of the sheep, of the dog and of the rat, is deprived entirely of its hæmolytic action for the cells with which it had been in contact. I shall have to refer to this experiment later on.

The work done by Kyes and Sachs in Germany has been almost entirely

carried out with cobra venom. These observers confirm in a general way the amboceptor nature of the working of cobra venom in the sense of Flexner and Noguchi. They, however, show that, while this poison can by itself hæmolyse the washed cells of certain species, it has no action on the washed cells of certain other species. Therefore, as regards the action of cobra venom the red cells of animals can be divided into two groups : namely, A, those which are sensitive to cobra venom alone; and B, those which are not dissolved by cobra venom alone. In group A are the red cells of the dog, the guinea-pig, man, the horse, etc. These cells vary in their degree of sensibility; thus the cells of the dog and of the guinea-pig are the most sensitive, while those of the horse are the least sensitive. In group B are the cells of the ox, of the sheep and of the goat. With these cells hæmolysis can be brought about by the addition of certain fresh sera; thus, guinea-pig's serum is able to act as complement for cobra venom and ox cells. These complements are destroyed by heating at 56°C. Further, they state that sheep's cells bind cobra venom to a certain extent, and that at 0° C. no complement is fixed at the same time. They, therefore, conclude that the amboceptor nature of cobra venom is evident and may be taken as proved.

In following up the results got with cobra venom and the washed cells of class A they show by a series of ingenious experiments that the reason why these cells, even when carefully and thoroughly washed, are sensitive to cobra venom alone, is that the lecithin contained within them is able to act as an activating substance. They prove that the lecithin is bound to the stroma of the cells, and that on dissolving red cells with distilled water it remains attached to the stroma; they offer as a probable explanation of the non-sensitiveness of the cells of class B to cobra venom the supposition that the lecithin contained in these cells is so firmly bound to the stroma that the venom cannot separate it from this binding. Kyes and Sachs in further elaborating these discoveries show that free lecithin prepared from the yolk of egg can act as complement for cobra venom and the cells which are not dissolved by the venom alone; they carefully compare the action of lecithin with that of serum complement and, while they show that there are several important differences in the conditions of working of these two substances, they are of opinion that the action of lecithin is of the same nature as that of serum complement. They also point out that Calmette's observation[3], namely, that certain normal sera are more suitable to cause hæmolysis with cobra venom after they have been heated than in the fresh condition, receives a simple explanation in the light of their observations. They show, in fact, that this thermostabile substance in serum which acts as a complement to cobra venom is nothing more or less than lecithin, which probably by the heating of the serum becomes loosened or entirely freed from its bindings, and which only then is in a condition to be taken up by the amboceptor of the poison.

In further pursuing the study of this complemental action of lecithin for cobra venom Kyes was able to prepare a new compound, a cobra venom lecithide. This substance acts as a powerful hæmolysing agent for all cells against which it was tested. Further, in the course of the preparation of this new compound the neurotoxic element of cobra venom was separated from the hæmolytic element, which latter completely joined on to the lecithin. He examined the properties of this lecithide and pointed out various differences between it and lecithin, and also between it and cobra venom amboceptor. Kyes was also able to prepare lecithides not only for several other snake poisons but also for the poison of the scorpion, the hæmolytic action of which poison is analogous to that of cobra venom.

With this short introduction, which sets forth the present position of the various problems of snake venom hæmolysis, we may pass on to the consideration of some further points which I have recently investigated. In the first place various other venoms were investigated as regards their action on the red cells of different species of animals, (*a*) on the washed cells alone, (*b*) with the addition of the homologous serum as complement, and (*c*) with the addition of free lecithin as complement. The poisons used were for the most part those of Indian snakes, both of the colubrine and viperine families. In the tables they are denoted by the initials of the genus and species as follows :—

I.—Colubridæ.

Naja tripudians (cobra)=N. T. V.
Naja bungarus (king cobra)=N. B. V.
Bungarus cæruleus (krait)=B. C. V.
Bungarus fasciatus (banded krait)=B. F. V.
Hoplocephalus curtus (tiger snake)=H. C. V.
Enhydrina valakadien (sea-snake)=E. V. V.

II.—Viperidæ.

Daboia Russellii (daboia)=D. R. V.
Echis carinata (phoorsa)=E. C. V.
Trimeresurus gramineus (green pit viper)=T. G. V.
Crotalus adamanteus (rattlesnake)=C. A. V.

The red cells employed were those of the dog, of the ox and of the goat. For the lecithin I have to thank Professor Ehrlich; it was prepared from the yolk of egg, was perfectly pure and was designated "Agfa" lecithin.

The following is a short account of the technique employed in all instances :—

The blood having been gently defibrinated was centrifugalised. The supernatant serum was carefully pipetted off and put aside for use as serum

complement. The red cells were then thoroughly washed 4 or 5 times with sterile salt solution (0·85 per cent.); being centrifugalised between each washing. Finally, a 5 per cent. suspension of the washed cells was made with 0·85 per cent. sterile salt solution; 1 c.c. of such a suspension was the amount used in each preparation of all the experiments.

The venom solutions were made up fresh each time as required, 0·85 per cent. sterile salt solution being the solvent.

A stock solution of lecithin of a strength of one per cent. was made in the purest methyl alcohol (Merck): from this solution a ten-fold dilution with sterile 0·85 per cent. salt was made as required. In all experiments 0·2 c.c. of this 0·1 per cent. solution was the amount added to each tube.

When serum was used as the activating agent, the amount added to each preparation was 0·5 c.c. of a 2-fold dilution of fresh serum. In each instance the total contents of each tube were made up with salt solution to 2 or 2·5 c.c. After the mixtures were prepared the tubes were kept at 37°C. for one hour and then placed in the ice chest overnight, when the results were recorded.

The first observations were made with the cells of the dog. Table I contains the results of these experiments with the venoms alone, that is to say, without the addition of any complemental substance. From this table it is seen that we can divide snake poisons into two groups according as they have or have not any hæmolytic action on dog's cells without the addition of free activating agent. Further, it is seen that the venoms which make up the first group, that is, those poisons which can hæmolyse

Table I.

Amount of venom in milligrammes.	Dog's washed cells: 1 c.c. of 5 per cent. suspension.									
	N. T. V.	N. B. V.	B. C. V.	B. F. V.	H. C. V.	E. V. V.	D. R. V.	E. C. V.	F. G. V.	C. A. V.
5	C. H.	No H.	C. H.	No H.	Slight.	No H.	C. H.	C. H.	No H.	No H.
1	C. H.	No H.	Trace	No H.	Slight.	No H.	C. H.	Nearly C. H.	No H.	No H.
0·5	C. H.	No H.	No H.	No H.	Trace.	No H.	C. H.	Trace.	No H.	No H.
0·1	C. H.	No H.	No H.	No H.	No H.	No H.	C. H.	No H.	No H.	No H.
0·05	C. H.	No H.	No H.	No H.	No H.	No H.	C. H.	No H.	No H.	No H.
0·01	Nearly C. H.	No H.	No H.	No H.	No H.	No H.	Nearly C. H.	No H.	No H.	No H.
0·005	Trace.	No H.	No H.	No H.	No H.	No H.	Well marked.	No H.	No H.	No H.
0·001	No H.	No H.	No H.	No H.	No H.	No H.	Mere trace.	No H.	No H.	No H.
0·0005	No H.	No H.	No H.	No H.	No H.	No H.	Mere trace.	No H.	No H.	No H.

C. H. = Complete hæmolysis.

dog's cells without the addition of extracellular complement, vary considerably in the degree of this action. Thus, cobra venom and daboia venom even in small amount have a complete hæmolysing effect; the venoms of Bungarus cæruleus and Echis carinata have a complete action only when used in large quantity, while the poison of Hoplocephalus curtus has only a slight effect even when present in comparatively large amount. Group 2, namely, those venoms which have no hæmolytic action on dog's washed cells, consists of the poisons of Naja bungarus, Bungarus fasciatus, Enhydrina valakadien, Trimeresurus gramineus and Crotalus adamanteus.

Turning now to table II, in which are set forth the results obtained with the same quantities of the same poisons as in the experiments recorded in table I but with the addition of serum complement, the homologous serum being used for this purpose, we find that all venoms have now a marked hæmolytic action and that with the exception of the venom of Enhydrina valakadien the quantitative

Table II.

Amount of venom in milligrammes.	Dog's washed cells: 1 c.c. of 5 per cent. suspension. Dog's serum: 0·5 c.c. of 2-fold dilution.									
	N. T. V.	N. B. V.	B. C. V.	B. F. V.	H. C. V.	E. V. V.	D. R. V.	E. C. V.	T. G. V.	C. A. V.
5	C. H.	C. H.	C. H.	C. H.	C. H.	Nearly C. H.	C. H.	C. H.	C. H.	C. H.
1	C. H.	C. H.	C. H.	C. H.	C. H.	Nearly C. H.	C. H.	C. H.	C. H.	C. H.
0·5	C. H.	C. H.	C. H.	C. H.	C. H.	Nearly C. H.	C. H.	C. H.	C. H.	C. H.
0·1	C. H.	C. H.	C. H.	C. H.	C. H.	Nearly C. H.	C. H.	C. H.	C. H.	C. H.
0·05	C. H.	C. H.	Nearly C. H.	C. H.	C. H.	Well marked.	C. H.	C. H.	C. H.	C. H.
0·01	C. H.	C. H.	Nearly C. H.	C. H.	Nearly C. H.	Trace.	C. H.	Marked.	C. H.	C. H.
0·005	Nearly C. H.	Marked.	Marked	Nearly C. H.	Nearly C. H.	Trace.	C. H.	Mere trace.	C. H.	C. H.
0·001	Marked.	Mere trace.	Trace.	No H.	Slight	No H.	Nearly C. H.	No H.	Slight.	Nearly C. H.
0·0005	Slight.	No H.	No H.	No H.	Trace.	No H.	Slight.	No H.	Trace.	Mere trace.

C. H.=Complete hæmolysis.

differences to be made out between the individual poisons are not great.

It is also to be noted that smaller amounts of both cobra and daboia poisons

Table V.

Amount of venom in milligrammes.	Goat's washed cells: 1 c.c. of 5 per cent. suspension. Lecithin: 0·2 c.c. of 0·1 per cent. solution.							
	N. T. V.	M. B. V.	B. C. V.	B. F. V.	E. V. V.	D. R. V.	E. C. V.	C. A. V.
5	Nearly C. H.	Slight	Mere trace.	Trace	Well marked.	C. H.	C. H.	No H.
1	Nearly C. H.	Mere trace.	Nearly C. H.	Trace	Mere trace	C. H.	C. H.	No H.
0·5	Well marked.	No H.	Nearly C. H.	Trace	No H.	C. H.	C. H.	No H.
0·1	Marked	No H.	Well marked.	Trace	No H.	C. H.	Well marked.	No H.
0·05	Marked	No H.	Well marked.	Trace	No H.	C. H.	Slight	No H.
0·01	Trace	No H.	Mere trace.	No H.	No H.	C. H.	No H.	No H.
0·005	Mere trace.	No H.	Mere trace.	No H.	No H.	Slight	No H.	No H.
0·001	No H.	No H.	No H.	No H.	No H.	No H.	No H.	No H.
0·0005	No H.	No H.	No H.	No H.	No H.	No H.	No H.	No H.

C. H. = Complete hæmolysis.

has no hæmolysing action whatsoever. The other poisons come in between these two extremes.

These observations, then, confirm and extend the observations of the previous workers mentioned above. They, also, offer a simple explanation of the discrepancies which seemed to exist between the American and the German results. For, it will be remembered that Flexner and Noguchi made the statement that no venom used by them had any hæmolytic action on the washed cells of any species with which they worked; Kyes and Sachs, on the other hand, showed that cobra venom alone, using the lecithin contained in the cells, had a marked action on the washed cells of various species, but had no effect on those of certain other species. It is apparent, therefore, that Flexner and Noguchi had not experimented with any of the combinations of venoms and washed cells in which hæmolysis takes place without the addition of complemental substance. Their experiments had evidently been confined to those venoms which have little affinity for lecithin, such as the venom of Crotalus adamanteus, and which, therefore, are not able to withdraw the lecithin from its binding to the stroma of the

cells even in the case of those cells, such as the cells of the dog, in which this binding is evidently loose.

In previous papers [4] I have pointed out that working with three pure antivenomous sera the cytophile groups of the amboceptors of snake venoms differ greatly amongst themselves; we have now proof of differences in the lecithin affinities of the various poisons, some poisons evidently being well provided with lecithinophile groups while others have few or none.

The question of the failure of both ox serum and goat serum to act as complements for any venom with the homologous cells is a question which has already been dealt with by Kyes and Sachs. It need not, therefore, be further discussed here.

While the experiments which have been tabulated above are in themselves of a certain interest and importance, they were in reality undertaken with an ulterior motive, namely, as a necessary preliminary to ascertaining what combinations of cells, venom and complement were suitable for further research into the conditions which underlie the union of these bodies. In the case of hæmolytic sera it is well known that under certain conditions the receptors of the red cells take up and fix the amboceptors of the serum. Ehrlich and Morgenroth [5] have shown that red cells might in certain instances take up many times the minimum hæmolytic dose of immune body, and that this union takes place at o°C. in presence of complement, which remains free. Thus a method is afforded for separating immune body from complement. Muir [6] has confirmed these observations. Further, working with ox corpuscles and rabbits' immune serum, which had been inactivated by heat, Muir found that the number of hæmolytic doses taken up by the cells varied from 6 to 10 when the mixtures were allowed to stand at 37°C. for 2 hours. All these experiments, whether made at o°C. with unheated serum or at 37°C. with heated serum, are of necessity of such a nature that either complement or a modification of complement is present. In the case of the experiments at o°C. with unheated serum free unmodified complement is present, and in the case of the experiments at 37°C. with heated serum the modification of complement which Ehrlich has designated complementoid still exists. It is, therefore, evident that all experiments which have had as their object the fixation of amboceptor to red cell have been made in the presence of either complement or modified complement. Now, it is quite conceivable that both these substances might act in such a way as to favour the combination between red cell receptor and cytophile group of immune body without themselves entering into the union. I know of no similar experiments in which the complement had been previously neutralised with an anti-complement, contained in a serum which had no complement for the combination of immune body and red cell which was being used; but even if such experiments did exist, the objection

might be raised that the anti-complement had only occupied the haptophore group of the complement, but had not altered its action as a stimulus to the union of red cell receptor and immune body.

Now, a moment's consideration will show that in the case of venom hæmolysis the objections which have been mentioned above cannot be brought forward. For in venom we have a pure amboceptor, which can be manipulated free from complement or any modification of complement. We have, therefore, to select only those instances in which the venom alone has no action on the washed cells, but has a marked hæmolytic effect on the addition of either serum complement or lecithin, in order to obtain a series of experiments in which the question of fixation of amboceptor can be investigated under the best conditions. Furthermore, with dried venoms, which retain their power indefinitely in this condition, and with a complemental substance such as lecithin, which can be kept without change in solution in methyl alcohol, we have conditions of experiment which allow of quantitative observations being made at intervals, which observations will be strictly comparable amongst themselves.

The combinations which were chosen for the present experiments were as follow:—

(1) Dog's cells—King cobra venom—Dog's serum complement.
(2) Dog's cells—Bungarus fasciatus venom—Dog's serum complement.
(3) Dog's cells—Crotalus adamanteus venom—Dog's serum complement.
(4) Dog's cells—Bungarus fasciatus venom—Lecithin.
(5) Ox cells—Cobra venom—Lecithin.
(6) Ox cells—Daboia venom—Lecithin.
(7) Goat's cells—Daboia venom—Lecithin.
(8) Goat's cells—Echis carinata venom—Lecithin.

A reference to the Tables 1 to 5 will show that in all these instances the venom alone had no action on the washed cells, but that on the addition of the complemental substance, serum or lecithin as the case might be, a complete hæmolytic effect was obtained, even when a relatively small amount of poison was used. The question, then, which was set to be answered was this:—How many hæmolytic doses of venom can a definite amount of red cells take up and fix in the absence of complemental substance? Eight series of experiments with the different combinations of cells, venom and activating agent, as mentioned above, were made with the object of solving this question. As the technique employed was exactly the same in each series, the description of only one observation, namely, that with ox cells, daboia venom and lecithin, will be given in detail.

The blood was gently whipped; it was then centrifugalised, and the red cells carefully and thoroughly washed to remove any trace of serum. A 5 per cent. suspension of the washed cells was then made in 0·85 per cent. sterile salt

solution ; of this suspension 1 c.c. was the amount used in all preparations. In the first instance the minimum complete hæmolysing dose of the venom was accurately determined for this quantity of red cell suspension when a definite amount of lecithin was added. This amount was 0·2 c.c. of a 0·1 per cent. solution, which was made with salt solution (0·85 per cent.) as required from the stock 1 per cent. solution in methyl alcohol. The contents of each tube were made up to 2 c.c. with salt solution. The tubes were then placed in the incubator (37°C.) for one hour and overnight in the ice chest. The results were then appraised. Such an estimation is shown in table VI. In this case it will be seen that the minimum complete hæmolysing dose was 0·008 milligramme of venom.

Table VI.

Amount of daboia venom in milligrammes.	Ox washed cells : 1 c.c. of 5 per cent. suspension. Lecithin : 0·2 c.c. of 0·1 per cent. solution.
	Result.
0·01	Complete hæmolysis.
0·009	Ditto.
0·008	Ditto.
0·007	Nearly complete hæmolysis.
0·006	Well marked „
0·005	Marked „
0·004	Slight „
0·003	Trace „
0·002	Trace „
0·001	Mere trace „
Nil (Control)	No hæmolysis.

Minimum complete hæmolysing dose = 0·008 milligramme.

This amount being determined, the next procedure was as follows :- To a number of tubes each containing 1 c.c. of a 5 per cent. suspension of ox washed cells were added increasing multiples of the minimum complete hæmolysing dose of venom, namely, 1, 2, 4, 6, 8, 10 and 20 doses. The contents of each tube were made up to 2 c.c. with salt solution. The tubes were then placed in the incubator at 37°C. for 2 hours, the contents being well shaken up from time to time. They were then thoroughly centrifugalised. (1) The supernatant fluid was carefully pipetted off from each tube into a fresh tube. To each of these latter tubes were added 1 c.c. of the 5 per cent.

suspension of ox cells and 0·2 c.c. of 0·1 per cent. solution of lecithin. The tubes were then placed in the incubator for one hour and overnight in the ice chest. (2) The sedimented red cells were thoroughly washed six times with salt solution, being centrifugalised between each washing. Then a suspension of these washed cells was made in each instance with 2 c.c. salt solution. Finally 0·2 c.c. of a 0·1 per cent. solution of lecithin was added to each tube. The tubes were kept as usual for one hour at 37°C. and overnight in the ice chest. The results of this experiment are given in Table VII.

Table VII.

Amount of Jaboia venom in milligrammes.	Number of complete hæmolysing doses.	Supernatant fluid + 1 c.c. of 5 per cent. ox cells + 0·2 c.c. of 0·1 per cent. Lecithin.	Sedimented red cells + 0·2 c.c. of 0·1 per cent. Lecithin.
0·16 . . .	20	Complete hæmolysis . .	No hæmolysis.
0·08 . . .	10	Ditto . .	Ditto.
0·064 . . .	8	Ditto . .	Ditto.
0·048 . . .	6	Ditto . .	Ditto.
0·032 . . .	4	Ditto . .	Ditto.
0·016 . . .	2	Ditto . .	Ditto.
0·003 . . .	1	Ditto . .	Ditto.
Nil (control) . .	*Nil.*	No hæmolysis . . .	Ditto.

The study of this table will at once show that in not a single preparation had any fixation of the amboceptor of the venom by the receptors of the red cells taken place. That this result cannot be explained on the supposition of the removal of the amboceptor from the cells by the repeated washing to which the latter were subjected, is evident from the fact that the supernatant fluid of the tube containing only a single hæmolysing dose was able to completely hæmolyse the same amount of fresh corpuscles. It is also noteworthy that even when a fairly large mulitiple, namely 20 times of the hæmolysing dose was used, still the receptors of the cells did not take up and fix any amboceptor.

The other experiments were made in exactly the same manner as that described above. In the case of those observations in which serum complement was used the amount of serum added to each tube was 0·5 c. c. of a two-fold dilution. In all these observations a similar result was obtained to that mentioned above, that is to say, in no case was there any anchoring of the venom amboceptor to the red cell. The following Table VIII shows at a glance the various combinations,

with the minimum complete hæmolysing dose of the poison, which gave these results.

Table VIII.

Corpuscles.	Venom.	Minimum complete hæmolysing dose.	Complemental substance.	Amount of complemental substance
Dog . . .	King Cobra . .	0·01 millig.	Serum . .	0·5 c.c. of 2-fold dilution.
Dog . . .	Bungarus fasciatus .	0·006 „	Serum . .	Ditto.
Dog . . .	Crotalus adamanteus	0·002 „	Serum . .	Ditto.
Dog . . .	Bungarus fasciatus .	0·006 „	Lecithin . .	0·2 c.c. of 0·1 % solution.
Ox . . .	Cobra . . .	0·05 „	Lecithin . .	Ditto.
Ox . . .	Daboia . . .	0·008 „	Lecithin . .	Ditto.
Goat . . .	Daboia . . .	0·01 „	Lecithin . .	Ditto.
Goat . . .	Echis carinata .	0·2 „	Lecithin . .	Ditto.

I have, therefore, experimented with six venoms in eight different combinations of cell, venom amboceptor and activating agent and have not found a single instance in which the amboceptor was taken up and fixed by the cells in the absence of complemental substance.

These results appear to be in direct opposition to those obtained by Flexner and Noguchi mentioned at the beginning of this paper. For these observers state that, "venom solution treated with dog, rabbit and guinea pig's washed corpuscles in succession gives up to each a part of its intermediary body. No one kind of corpuscle is capable of fixing the entire content of intermediary bodies." They, however, do not mention the venom or venoms with which these observations were made nor do they give any details of the experiments. In a later paper, however, they state that water mocassin venom was deprived of its entire hæmolytic action for the cells of the sheep, the dog and the rat, respectively, by keeping these cells in contact with 1 per cent. solution of the venom in the cold (0°C.) for three hours. In these experiments it is not stated that washed corpuscles were used. On the other hand, from the fact that the blood cells and venom were kept in contact at 0°C. it is to be presumed that unwashed corpuscles were used, that is to say, that serum complement was present. If this was the case, these experiments would be open to the same objection which, we have seen, applies to similar observations with hæmolytic sera. It is evident, therefore, that neither of the observations made by Flexner and Noguchi is comparable, either as regards exactness of detail or as regards similarity of technique, with those which I have brought forward above.

Kyes states that sheep's cells can bind cobra venom only very slightly; but here again no details of the experiments are given. Further, he states that at o°C. there is no fixation of cobra venom in weak solution by ox cells, an observation which is in keeping with those which I have now brought forward. In all the work, however, on venoms emanating from Ehrlich's laboratory no experiments at all comparable to those collated above have been detailed.

The answer, then, to the question which we set ourselves is, that under certain conditions of experiment and in the absence of complemental substance the red cell receptor is incapable of taking up and anchoring any venom amboceptor: this latter is found intact in the supernatant fluid after centrifugalisation. Such a result is, as we have pointed out, different from that obtained with hæmolytic sera, when these are tested in the presence of complement or complementoid. One example, however, of the failure of the red cell receptor to take up the hæmolytic amboceptor is given by Ehrlich and Sachs[7]. Using guinea-pig's washed cells, inactivated ox serum and fresh normal horse serum, these workers found that, if the inactivated ox serum and cells were kept in contact for one hour at 37°C. and if then the supernatant fluid was separated from the cells by centrifugalisation, nearly the total amount of amboceptor of the ox serum could be demonstrated in the supernatant fluid, while only a trace had been taken up by the red cells. I know of no bacteriolytic observations in which such a phenomenon has been described. We can conclude then, that as far as snake venoms are concerned, the hæmolytic amboceptor is not taken up by the red cell in the absence of complemental substance. If, therefore, the process of venom hæmolysis is strictly analogous to serum hæmolysis and to bacteriolysis, we should expect to find that the amboceptor in both these instances does not join on to the receptor of the cell under the same conditions, that is, in the absence of complement or modified complement. Such experiments are in progress.

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BY

CAPTAIN GEORGE LAMB, M.D., I.M.S.

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